EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

Interindividual Variability: New Ways to Study and Implications for Decision-Making

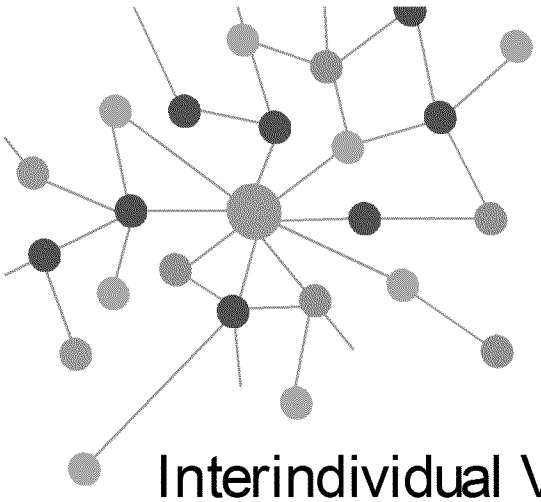
September 30-October 1, 2015

National Academy of Sciences,
Engineering, and Medicine
Room 100, Keck Center
500 5th St. NW, Washington, DC

EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

AGENDA BOOK CONTENTS

A. AGENDA



AGENDA

Interindividual Variability: New Ways to Study and Implications for Decision Making

SEPTEMBER 30-OCTOBER 1, 2015 ▫ WEDNESDAY 9:00–4:35, THURSDAY 8:30–NOON

THE NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE,

KECK CENTER, 500 FIFTH STREET NW, ROOM 100, WASHINGTON, DC

THIS WORKSHOP WILL BE WEBCAST

INTERINDIVIDUAL VARIABILITY REFERS TO THE RANGE OF DIFFERENCES AND DEGREES in which people respond to environmental stressors. This variation of response within populations has long been a key consideration by those tasked with risk-based decisions. These variations can be intrinsic (e.g., heritable characteristics), extrinsic (e.g., stress), plastic (e.g., body weight), and static (e.g., genetics).

Since 2012, when the Committee first held a workshop on *Individual Variability and the Biological Factors that Underlie Individual Susceptibility to Environmental Stressors and Their Implications for Decision-Making,* the scientific tools aimed at eluci - dating the sources of this variation have advanced. Scientific tools such as in vitro toxicology methods using highly diverse cell lines, in vivo methods using highly diverse animal populations, and epidemiologic analytical approaches which explore mediators within the causal pathway can all help decision makers better understand intrinsic, extrinsic, plastic, and static, sources of interindividual variability.

This workshop will discuss the scientific tools and their application within decisions contexts such as setting regulations, determinations of hazard levels, and decisions about the safety of new chemicals. Workshop participants will leave with a better under - standing of these new tools and how they may be used to advance the science behind risk-based decisions.

WEDNESDAY, SEPTEMBER 30, 9:00 AM–4:35 PM

9:00 **Welcome**

9:15 **The Importance of Understanding Interindividual Variability in Response to Chemical Exposures—Linda Birnbaum,** National Institute of Environmental Health Sciences

9:45 **Introduction: Why Interindividual Variability Matters in Decision Contexts—Lauren Zeise†,** California EPA, Office of Environmental Health and Hazard Assessment

10:10 *Break*

10:25 **How Interindividual Variability is Captured in Environmental Regulations—John Vandenberg,** EPA–National Center for Environmental Assessment

10:50 **How Interindividual Variability is Addressed When Considering Pharmaceutical Safety—Jon Cook,** Pfizer

11:10 **How Interindividual Variability is Captured in Occupational Guidelines—Terry Gordon,** American Conference of Governmental Industrial Hygienists Threshold Limit Values Committee

11:35 *Lunch—Room 106 is reserved for committee, speakers, and liaisons*

(continued)

† Indicates a member of the Standing Committee on Use of Emerging Science for Environmental Health Decisions.

WEDNESDAY, SEPTEMBER 30, CONTD.

Session 1 In Vitro Methods and Resources

12:35 **Moderator: Ivan Rusyn†,** Texas A&M University

12:40 **1000 Genomes High-Throughput Screening Study—Fred Wright,** North Carolina State University

1:20 **NIH Roadmap Epigenomics Program: Resources, Obstacles, and Opportunities—John Satterlee,** National Institute on Drug Abuse

1:50 **Integrating In Vitro and In Silico Methods to Evaluate Variability—Barbara Whetmore,** The Hamner Institutes for Health Sciences

2:20 **Panel Discussion—How In Vitro Methods and Resources May Improve Risk Decisions**

- **John Vandenberg**
- **Anna Lowit,** EPA–Office of Pesticide Programs
- **Michael Pacanowski,** FDA–Center for Drug Evaluation and Research
- **Jon Cook**
- **Gary Ginsberg,** Connecticut Department of Public Health
- **Terry Gordon**
- **Fred Wright**
- **John Satterlee**
- **Barbara Whetmore**

2:50 *Break*

Session 2 In Vivo Methods

3:20 **Moderator: Lauren Ziese†**

3:25 **Collaborative Cross—David Threadgill,** Texas A&M University

3:55 **Diversity Outbred Mice—Michael Devito,** NIEHS–National Toxicology Program Laboratory

4:25 **Panel Discussion—How In Vivo Methods May Improve Risk Decisions**

- **John Vandenberg**
- **Anna Lowit**
- **Michael Pacanowski**
- **Jon Cook**
- **Gary Ginsberg**
- **Terry Gordon**
- **David Threadgill**
- **Michael Devito**

5:00 **Adjourn for the Day**

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THURSDAY, OCTOBER 1, 8:30AM–12:00PM

Session 3 Epidemiologic Methods

8:30 **Moderator: John Balbus,** National Institute of Environmental Health Sciences

8:35 **Epidemiologic Techniques to Evaluate Factors Associated with Interindividual Variability—Joel Schwartz†,** Harvard School of Public Health

9:00 **Machine Learning Techniques to Evaluate Interindividual Variability—Joshua Millstein,** Keck School of Medicine of USC

9:30 **Panel Discussion— How New Epidemiology Methods May Improve Risk Decisions**

- **John Vandenberg**
- **Anna Lowit**
- **Michael Pacanowski**
- **Jon Cook**
- **Gary Ginsberg**
- **Joel Schwartz†**
- **Joshua Millstein**

10:00 *Break*

Session 4 Implications of Understanding Interindividual Variability

10:30 **Panel Discussion**

Moderator: Gina Solomon, California Environmental Protection Agency

- **Kimberly White,** American Chemistry Council
- **Michael Yudell,** Drexel University
- **Richard Denison†,** Environmental Defense Fund
- **James C. O'Leary,** Genetic Alliance

11:45 **Closing Remarks—Lauren Zeise†**

12:00 **Adjourn Workshop**

12:30 **Committee and liaisons meet in Room 106**

For more information and to subscribe for updates, please visit http://dels.nas.edu/envirohealth

Emerging Science meetings are free and open to the public.

About the Committee

At the request of the National Institute of Environmental Health Sciences (NIEHS), the National Academy of Sciences formed the Standing Committee on Use of Emerging Science for Environmental Health Decisions to facilitate communication among government, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.

AGENDA FOR COMMITTEE AND GOVERNMENT LIAISONS

September 30- October 1, 2014
Keck Center - 500 5th Street, NW, Washington, DC

Wednesday, September 30

9:00 AM – 5:15 PM **Interindividual Variability: New Ways to Study and Implications for Decision Making Meeting**
Keck 100

5:45 PM – 7:30 PM **Dinner at Bistro D'OC (518 Tenth St, NW, Washington, DC)**
Please bring cash to cover your dinner (~$36 for dinner, not including drinks)

Thursday, October 1

8:30 AM – 12:00 PM **Interindividual Variability: New Ways to Study and Implications for Decision Making Meeting Continues**
Keck 100

12:00 PM – 12:30 PM Break. Committee members and liaisons, please obtain your lunch on 3rd floor and return to Room 106.

Open Session

12:30 PM – 2:30 PM **Committee and Liaison Meeting**
Keck 106

12:35 PM Welcome and Announcements

12:45 PM Discussion of Interindividual Variability Meeting

1:20 PM Discussion of Meeting on *Microbiome Functions Related to Environmental Health* (January 14-15, 2015; handout to be provided)

2:00 PM Brainstorming about Potential Future Meeting Topics
We welcome your submission of specific topics to the staff in advance. Topics might include: emerging areas of science, new tools or approaches, and/or environmental health-related challenges faced by your agency.

2:30 PM **Committee and Liaison Meeting Adjourns**

B. WORKSHOP

Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S., is the Director of the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH), and Director of the National Toxicology Program. A board certified toxicologist, Birnbaum has served as a federal scientist for over 35 years. Dr. Birnbaum has received many awards and recognitions, including the Women in Toxicology Elsevier Mentoring Award, the Society of Toxicology Public Communications Award, EPA's Health Science Achievement Award and Diversity Leadership Award, the National Center for Women's 2012 Health Policy Hero Award, Breast Cancer Fund Heroes Award, and 14 Science and Technology Achievement Awards, which reflect the recommendations of EPA's external Science Advisory Board, for specific publications. Dr. Birnbaum was also elected to the Institute of Medicine of the National Academies, and received an honorary degree from Ben-Gurion University in Israel. Dr. Birnbaum is a former president of the Society of Toxicology, the largest professional organization of toxicologists in the world; former chair of the Division of Toxicology at the American Society of Pharmacology and Therapeutics; and former vice president of the American Aging Association. She is the author of more than 700 peer-reviewed publications, book chapters, and reports. She is also an adjunct professor at several universities, including the University of North Carolina at Chapel Hill and Duke University. A native of New Jersey, Dr. Birnbaum received her M.S. and Ph.D. in microbiology from the University of Illinois at Urbana-Champaign.

Jon C. Cook, Ph.D., is Senior Director of Investigative Toxicology at Pfizer Inc. (1998-present). He is located in Groton, CT and leads the Investigative Toxicology group that de-risks findings observed in nonclinical studies. He has worked at Pfizer for 16 years on early and late-stage drug development teams. Jon worked with Searle colleagues to obtain approval for Celebrex and Valdecoxib. Jon later worked on the team to register Lasofoxifene and led de-risking efforts following complete response letters. More recently, he was a member of the team working on Lyrica de-risking of hemangiosarcoma to obtain the Generalized Anxiety Disorder indication. He currently leads a Drug Safety team of scientists to implement a Precision Medicine strategy for his line and is and a member of Drug Safety's Science and Technology Board. Prior to joining Pfizer Inc., he was a Senior Research Toxicologist at DuPont-Haskell Laboratory (1987-1998) and a Postdoctoral Fellow at Chemical Industry Institute of Toxicology (1985-1987). Dr. Cook received his B.S. in Physiology from the University of California, Davis, and his M.S. and Ph.D. degrees in Toxicology from North Carolina State University. He is a Diplomate of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences. He served on the Editorial Boards of the Journal of Toxicology and Environmental Health (1988-1994), Fundamental & Applied Toxicology (1995-1998) and Toxicological Sciences (1998-2002). Dr. Cook received the Rutgers University Robert A. Scala Award in Toxicology in 1998.

Mike DeVito, Ph.D., is the acting Chief of the NTP Laboratory in the Division of National Toxicology Program at the National Institute of Environmental Health Sciences. From 1995 to 2002. Dr DeVito was a principle investigator in the Pharmacokinetics Branch of the National Health and Environmental Effects Research Laboratory at the US Environmental Protection Agency. From 2002-2009 he was Chief of the Pharmacokinetic Branch. Dr DeVito was one of the lead health effects researchers on the Dioxin Reassessment from 1991-2009. In 2009, Dr. DeVito joined the National Toxicology Program at NIEHS as the discipline leader for pharmacokinetic modeling. Dr DeVito's research has focused on the toxicity of persistent organic pollutants, thyroid hormone disruptors and pyrethroid pesticides. In addition, he has developed quantitative models to understand the exposure, dose and toxicity continuum for individual environmental chemicals as well as for cumulative risk assessments. He is presently the co-chair of the targeted testing working group for the Tox21 initiative at NTP, which is developing second tier tests as follow up studies for high-through put screening data. In addition he has interests in using HTS data to better understand the potential hazards and risks associate with chemical mixtures and natural products.

Gary Ginsberg, Ph.D., is a toxicologist and risk assessor for the Connecticut Department of Public Health and also has adjunct faculty positions at Yale and the University of Connecticut Health Center. He has served on several national panels including USEPA's Science Advisory Board, USEPA's Children's Health Protection Advisory Committee, the National Research Council's panels on USEPA risk methods (produced "Science and Decisions"), human biomonitoring, and most recently arsenic. He has published in the areas of chemical carcinogenesis, children's toxicokinetics, genetic polymorphisms, development of fish consumption advisories and a variety of other risk assessment topics. His professional experience has included working within the pesticide industry, consulting, academia and currently in state government.

Terry Gordon, Ph.D., directs a number of ongoing research projects that study the underlying toxicity of inhaled particles and gases encountered in ambient and occupational environments. The majority of his current research focus is on the adverse health effects of size-fractionated ambient particles and nanoparticles. He has examined the pulmonary effects of numerous inhaled particles in cell and rodent test models as well as in human subjects in panel studies. Dr. Gordon has sampled ambient particles across the U.S., Europe, and China to study the contribution of source and components to particle toxicity. Recently, he has collected particles in urban and rural environments in the NYC metropolitan area and in the Central Valley of California. These studies have found important differences in the toxicity of urban and rural particles and may have important impact on revisions to federal policies and regulations. Dr. Gordon is currently collaborating on a clinical study that evaluates the adverse cardiopulmonary effect of traffic-related pollution while exercising alongside the George Washington Bridge (car and diesel traffic) and the Garden State Parkway (car traffic only). Additional urban clinical studies are being planned to study the adverse effects of mainstream and second hand hookah smoke encountered in hookah lounges in NYC, as well as the assessment of particle exposure in taxi cabs and the NYC subway system. He is also interested in the interaction between inhaled pollutants and susceptibility factors and has broadened his particle research to examine age-related and genetic differences in response. Dr. Gordon is currently Chair of the Threshold Limit Value Committee of ACGIH, a committee that develops occupational exposure guidelines that protect workers' health around the world. He is the co-director of the Department of Environmental Medicine's inhalation exposure facility, one of the largest academic facilities of its kind in the country. Dr. Gordon has mentored numerous graduate students over the last 25 years (both MS and PhD students). He teaches the Environmental Sampling course at NYU, has been a member of the Department's Graduate Steering Committee for

over a decade, and serves as the Director of NYU's T32 training grant from NIEHS. Thus, overall, he has the necessary experience to participate in the proposed inhalation toxicology experiments.

Anna B. Lowit, Ph.D., received her Ph.D. in Environmental Toxicology from the University of Tennessee in 1998 where she was a Graduate Fellow in Sustainable Waste Management. Dr. Lowit began her career with EPA in 1998 with the Office of Pesticide Programs, where she remains today. Dr. Lowit is currently a Senior Scientist in the Health Effects Division where she advices senior managers and leads multidisciplinary teams on a variety of cross-cutting topics. She is currently one of the Co-Chairs of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM is composed of representatives from 15 U.S. Federal regulatory and research agencies that require, use, generate or disseminate toxicological and safety testing information and whose purpose is to promote and facilitate the 3Rs of toxicity testing (reduce, refine, replace) in regulatory toxicity testing. Dr. Lowit has extensive experience in developing cumulative risk assessments for groups of pesticides which share a common mechanism of toxicity (e.g., organophosphates, N-methyl carbamates). She also has interest in the integration of science along multiple lines of evidence (epidemiology, in vivo & in vitro experimental toxicology). She has particular interest in improving the use of quantitative approaches in human health risk assessment such as use of meta-analysis in deriving benchmark dose estimates and linking PBPK models with probabilistic exposure models.

Joshua Millstein, Ph.D., is Assistant Professor in the Division of Biostatistics at the University of Southern California. During his PhD work, and throughout his career, Dr. Millstein's research interests and efforts have focused on problems of high dimensional data, particularly population based genomic and transcriptomic data in the context of complex diseases. This work has included statistical methods development for the analysis of genomic data in the context of animal model, epidemiological, and clinical studies. During his time at Rosetta Inpharmatics in Eric Schadt's Genetics department he was one of two principle statistical geneticists to develop and apply an analytic approach for Merck's first genome-wide pharmacogenetic study of treatment effects and adverse events for a phase III clinical trial of Taranabant (MK-0364), an obesity drug. Areas of statistical methods development have included statistically powerful and computationally efficient approaches designed for epistasis, eQTL, causal inference, false discovery rates, and copy number alterations in tumor tissue. Currently, he is branching out and exploring analytic approaches for the microbiome and high order interactions between multiple drugs and between drugs and patient characteristics such as age, weight, gender, genetic background, and environmental exposures.

James O'Leary, MBA, is Chief Innovation Officer at Genetic Alliance. In his role, James works to foster innovation at Genetic Alliance and within its network of patients, hospitals, companies, universities, and government agencies. Over the past 10 years, James has built collaborations between these diverse stakeholders to seed change within the healthcare system and help individuals, families, and communities reclaim control of their health. He has harnessed health information and web technologies to enhance patients' ability to access information and use that information to make better decisions. In addition, he has worked with national public health systems, disease-specific organizations, and community groups to improve access to genetic services, engage consumers in national policy-setting, and institute legislation that protects the public from discrimination. James earned an MBA from the Wharton School of the University of Pennsylvania and a BS in Biology, concentrating in Cellular and Molecular Biology and Genetics from the University of Delaware. Prior to joining Genetic Alliance, James worked with PA Victory '04 supporting the John Kerry campaign.

Michael Pacanowski, Pharm.D., M.P.H., is the Associate Director for Genomics and Targeted Therapy in the Office of Clinical Pharmacology at FDA. His team of translational scientists is charged with advancing

the use of genomic and other biomarker innovations to maximize individualization in drug development. To that end, Dr. Pacanowski oversees a program focused on reviewing investigational new drugs, developing policies and processes, engaging stakeholders, and conducting regulatory science research. Dr. Pacanowski received his Pharm.D. from the Philadelphia College of Pharmacy and his M.P.H. from the University of Florida. He completed a residency in clinical pharmacology at Bassett Healthcare in Cooperstown, NY, and a clinical research fellowship in cardiovascular pharmacogenomics at the University of Florida.

John Satterlee, Ph.D., earned a B.S. in Biology from Cornell University and a M.S. in Science Education from Syracuse University. He completed a Ph.D. at the University of Wisconsin-Madison in plant molecular biology. His post-doctoral work at Brandeis University was in behavioral genetics. In 2003, Dr. Satterlee became co-director of the C. elegans Core facility at Massachusetts General Hospital where he identified new genes involved in a variety of developmental processes. In 2005 he began work at the National Institute on Drug Abuse. He has been co-coordinator of the Roadmap Epigenomics Program since its inception and is involved with other Common Fund programs including the 4D Nucleome and exRNA Communication Programs.

Joel Schwartz, PhD, is a Professor of Environmental Epidemiology at the Harvard School of Public Health and Director of the Harvard Center for Risk Analysis. His work has been instrumental in the removal of lead from gasoline, and the setting of particulate air pollution standards around the world. Schwartz's work tightened federal clean-air standards and improved compliance within industry. In addition to his research into lead, he was among the first to link elevated death rates to particulates of sulfur from coal-burning power plants and black carbon from motor-vehicle exhaust. Dr. Schwartz's current research interests include health consequences of exposure to pollutants, health effects of ozone exposure, and effects of antioxidants on respiratory health. Dr. Schwartz received his Ph.D. from Brandeis University.

Gina Solomon, M.D., M.P.H., is the Deputy Secretary for Science and Health at the California Environmental Protection Agency (CalEPA) and a Clinical Professor of Medicine at the University of California San Francisco (UCSF). Prior to coming to CalEPA in 2012, she was a senior scientist at the Natural Resources Defense Council, the director of the occupational and environmental medicine residency program at UCSF, and the co-director of the UCSF Pediatric Environmental Health Specialty Unit. Dr. Solomon's work has spanned a wide array of areas, including pediatric vulnerabilities in risk assessment, reproductive toxicity, and evaluating the use of novel data streams to screen chemicals for toxicity. She has also done work in exposure science for air pollutants, pesticides, mold, and metals in soil. She was involved in the response and aftermath of Hurricane Katrina, the Gulf oil spill, and the Chevron Richmond explosion and fire, and she is interested in the health effects of climate change. Dr. Solomon serves on the U.S. EPA's Science Advisory Board and Board of Scientific Counselors. She is also on the NAS Board on Environmental Studies and Toxicology, and previously served on the Committees on Toxicity Testing in the 21st Century and Exposure Science in the 21st Century. Dr. Solomon received her bachelor's from Brown University, her M.D. from Yale, and did her residency and fellowship training in internal medicine and occupational and environmental medicine at Harvard.

David W. Threadgill, Ph.D., is the Director of the recently formed Texas A&M Institute for Genome Sciences and Society at Texas A&M University. He is holds the title of University Distinguished Professor with a joint appointment in the Department of Veterinary Pathobiology in the College of Veterinary Medicine & Biomedical Sciences and the Department of Molecular and Cellular Medicine in the College of Medicine, where he also holds the Tom and Jean McMullin Chair of Genetics. Dr. Threadgill graduated with a bachelor of science degree in zoology from Texas A&M University in 1983 and earned a Ph.D. in

genetics from Texas A&M University in 1989. Dr. Threadgill subsequently held a National Institutes of Health Individual Postdoctoral Fellowship at Case Western Reserve University. In 1996, Dr. Threadgill joined Vanderbilt University as an assistant professor of Cell Biology and in 2000 moved his research laboratory to the newly formed Department of Genetics at the University of North Carolina at Chapel Hill where he was granted tenure and progressed to full professor. Dr. Threadgill moved to North Carolina State University in 2008 as Professor and Head of the Department of Genetics where he remained until being recruited back to Texas A&M University in 2013 to establish the Texas A&M Institute for Genome Sciences and Society. Dr. Threadgill was also a Visiting Distinguished Scientist at Oak Ridge National Laboratory form 2006-2008. Dr. Threadgill's research program uses the mouse as an experimental genetic model to investigate genetic and environmental factors that contribute to inter-individual differences in health and susceptibility to disease. His research program and trainees have been supported by the National Institutes of Health, Department of Defense, National Science Foundation, March of Dimes, Jimmy V Foundation, American Cancer Society, and the Kleberg Foundation.

John Vandenberg, Ph.D., is Director of the Research Triangle Park Division of the National Center for Environmental Assessment at the US Environmental Protection Agency. He is responsible for leadership, planning and oversight of EPA's Integrated Science Assessments for the major (criteria) air pollutants and Integrated Risk Information System (IRIS) assessments for high priority hazardous air pollutants. He began working at EPA in 1984, and was responsible for performing national-scale exposure and health risk assessments for numerous hazardous air pollutants. Following a year on assignment from EPA to the State of California to help develop risk assessment guidelines, he joined EPA's Office of Research and Development as Director of EPA's Research to Improve Health Risk Assessments program. He served in recent years as EPA's first National Program Director for particulate matter research and as acting director of EPA's Human Studies Division, and Experimental Toxicology Division. In recent years Dr. Vandenberg was Associate Director for Health at NCEA, where he had oversight responsibilities for much of EPA's health risk assessment activities. Dr. Vandenberg has been a consultant to the World Health Organization and has represented EPA in scientific meetings in Europe, South America, Africa and Asia, and he serves on numerous scientific advisory committees. In 2006, he was elected a Fellow of the Society for Risk Analysis. He is an adjunct professor at the Nicholas School of the Environment at Duke University and since 1991 he has taught a graduate-level course in air quality management. He received his B.A from the College of Wooster, Ohio, and the MS and PhD from Duke University in biophysical ecology.

Barbara Wetmore, Ph.D, is a Senior Research Investigator at The Hamner Institutes for Health Sciences. Her research interests focus on integrating predictive modeling tools with high-throughput screening and other in vitro strategies to address issues of importance in chemical and drug safety and risk assessment. Other research interests have focused on the application of genomic and proteomic tools to inform chemical mode of action assessments and biomarker discovery. She is currently vice-president-elect of the Society of Toxicology's In Vitro and Alternative Methods Specialty Section and has served as a study section reviewer for the US EPA and as an expert for the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM). Dr. Wetmore received her Ph.D. in Toxicology from North Carolina State University.

Kimberly Wise White, Ph.D., is a Senior Director in the American Chemistry Council Chemical Products and Technology Division. She possesses B.S. and M.S. degrees in biology and a Ph.D. in environmental toxicology. For the past several years, Dr. Wise has been actively involved in the management of scientific research and regulatory advocacy programs related to human health and toxicology. She

regularly engages with local, state, federal and international entities to promote utilizing the most relevant and up to date science information in human health risk assessments. She has also created and managed environmental sustainability, compliance, process safety and risk management programs.

Fred Wright, Ph.D., joined North Carolina State University in August 2013 as a Chancellor's Faculty Excellence Program cluster hire in Bioinformatics, and Professor in the Departments of Statistics and Biological Sciences. Wright is an internationally-known statistical geneticist, with wide-ranging interests including genomic bioinformatics, toxicogenomics, and the statistical principles underlying high-dimensional data analysis. Wright was recruited to be the new Director of the Bioinformatics Research Center (BRC), which has a strong history of research and training in statistical, evolutionary, and computational methods applied to a variety of genomic problems. Bioinformatics and computation have become central to much of biology, and Wright will lead the expansion of the BRC's focus to additional cross-cutting activities in human health and complex systems, while retaining the longstanding strengths of the BRC. Prior to joining NCSU, Wright was a Professor of Biostatistics at UNC Chapel Hill and member of the Lineberger Cancer Center and Carolina Center for Genome Sciences. He has been principal investigator of numerous grants, with activities ranging from development of new methods of gene mapping to expression-quantitative trait (eQTL) mapping for multiple tissues (credit christian). He was also principal investigator of an EPA-funded STAR Center to apply genomics principles to long-standing problems in toxicology. Wright is one of the lead investigators in the International Cystic Fibrosis Genetic Modifier Consortium, seeking to unravel the unexpected complexities of this disease, which was once thought to be "simple" in its underlying genetics. While at UNC, Wright fostered the development of a new statistical genetics curriculum, producing one of the most varied and rigorous programs among departments of Biostatistics. He is an elected Fellow of the American Statistical Association and the Delta Omega Honor society for Public Health. He received a B.A. in Statistics and Psychology from the University at Buffalo, and a Ph.D. in Statistics from the University of Chicago.

Michael Yudell, Ph.D., is Associate Professor and Interim Chair for the Community Health and Prevention Program at Drexel University's School of Public Health. Prior to joining Drexel in 2004, Michael Yudell held the positions of researcher in the Molecular Laboratories at the American Museum of Natural History, New York, where his work focused on genome policy and ethics, and the position of Health Policy Analyst at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, where he worked closely with the Institutes ethicist and deputy director on human genome project policy. Dr. Yudell is the author with Rob DeSalle of Welcome to the Genome: A User's Guide to the Genetic Past, Present, and Future, published in September 2004 by John Wiley and Sons. Yudell and DeSalle also edited The Genomic Revolution: Unveiling The Unity Of Life, published in 2002 by the Joseph Henry Press of the National Academy of Science. His work has also been published in Nature Reviews Genetics, The Journal of the History of Biology, Genome Technology, Natural History, and American Scientist. Dr. Yudell's work seeks to document historically stigmatized populations, the challenges they face in public health and medicine, and how this history impacts contemporary health challenges. His next book Race Unmasked: A 20th Century Struggle to Define Human Difference (forthcoming, Columbia University Press) a history that examines the way in which biologists and geneticists shaped the race concept during the 20th century from eugenics to the sequencing of the human genome. The book pays careful attention to the ways in which scientific conceptions of human difference impact both public health and medicine. Additionally, the work has important implications for bioethics and public health ethics given race's role in patient care and in our understandings of the

health of populations. He is also beginning work on a project that examines ethical issues associated with autism spectrum disorders, including risk communication and health disparities.

Lauren Zeise, PhD, Chief, Reproductive and Cancer Hazard Assessment Branch, of the California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment. In that role she oversees a variety of scientific activities concerning risk assessment, including chemical hazard and dose response assessment and development of improved methods for risk assessment. As part of Cal/EPA's environmental justice work, her group is also developing the Agency's approach to cumulative impact assessment – for characterizing the impact on communities of multiple sources of pollution and non-chemical stressors in the presence of community vulnerability. Her group works with other departments in California government in operating Biomonitoring California, the state's biomonitoring program. She co-led the team that developed California's Green Chemistry Hazard Trait regulation. Dr. Zeise has served on numerous national and international science advisory committees and boards focusing on environmental public health and improving the way chemicals are tested or evaluated for health risk. She has coauthored a number of National Academy of Science (NAS) reports, including "Science and Decisions: Advancing Risk Assessment" (2009), "Toxicity Testing in the 21st Century: A Vision and Strategy" (2007), "Sustainability and the US EPA" (2011), and "Understanding Risk: Informing Decisions in a Democratic Society" (1996). She is currently a member of the NAS committees including the Committee on Use of Emerging Science for Environmental Health Decisions. She is member, fellow, former editor and former councilor of the Society of Risk Analysis and was the 2008 recipient of the Society's Outstanding Risk Practioner Award. She is a lifetime NAS National Associate. She received her doctorate from Harvard University.

EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS
Interindividual Variability: New Ways to Study and Implications for Decision Making

Suggested Readings

**Available on the Interindividual Variability Google Drive (Readings will be deleted from drive on October 10th):*
https://drive.google.com/open?id=0B6Pq0IFe5Gy4Mk9NN29rNjRBSEk

General References

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Addressing Human Variability in Next-Generation Human Health Risk Assessments of Environmental Chemicals

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Background: Characterizing variability in the extent and nature of responses to environmental exposures is a critical aspect of human health risk assessment.

Objective: Our goal was to explore how next-generation human health risk assessments may better characterize variability in the context of the conceptual framework for the source-to-outcome continuum.

Methods: This review was informed by a National Research Council workshop titled "Biological Factors that Underlie Individual Susceptibility to Environmental Stressors and Their Implications for Decision-Making." We considered current experimental and analytical approaches, and emerging data streams (such as genetically defined human cell lines, genetically diverse rodent models, human omic profiling, and genome-wide association studies) that are providing new types of information and models relevant for assessing individual variability for application to human health risk assessments of environmental chemicals.

Discussion: One challenge for characterizing variability is the wide range of sources of inherent biological variability (e.g., genetic and epigenetic variants) among individuals. A second challenge is that each particular pair of health outcomes and exposures involves combinations of these sources, which may be further compounded by extrinsic factors (e.g., diet, psychosocial stressors, other exogenous chemical exposures). A third challenge is that different decision contexts present distinct needs regarding the identification—and extent of characterization—of individual variability in the human population.

Conclusions: Despite these inherent challenges, opportunities exist to incorporate evidence from emerging data streams for addressing individual variability in a range of decision-making contexts.

Key words: environmental agents, genetics, human health risk assessment, modeling, omics technologies, susceptible populations, variability. *Environ Health Perspect* 121:23–31 (2013). http://dx.doi.org/10.1289/ehp.1205687 [Online 19 October 2012]

Human variability underlies differences in the degrees and ways in which people respond to environmental chemicals, and addressing these differences is a key consideration in human health risk assessments for chemicals [Guyton et al. 2009; Hattis et al. 2002; National Research Council (NRC) 2009]. A large array of possible health outcomes is of concern for such assessments, and many sources of variation can influence the severity and frequency of the adverse effects at different exposure levels. These sources may be intrinsic (e.g., heritable traits, life stage, aging), or extrinsic, exogenous, and acquired (e.g., background health conditions, co-occurring chemical exposures, food and nutrition status, psychosocial stressors). Interactions between inherent and extrinsic factors create the large range of biological variation exhibited in response to a chemical exposure (NRC 2009). Given that biological variability in susceptibility is context-dependent, so too is the extent to which it needs to be described and quantified to inform any particular environmental decision. The salience of variability information for specific choices is affected by the range of available risk management options; the regulatory authority; the available time, resources, and expertise to collect data and conduct analyses; and stakeholder concerns.

Over the past decade, efforts to systematically "map" human variability have expanded dramatically, focusing mainly on genetic variation (Schadt and Björkegren 2012). In addition to genetic differences, omics studies have examined the impact of epigenetic, transcriptomic, proteomic, and metabolomic variation on disease susceptibility, prognosis, or options for pharmacotherapy (Chen et al. 2008; Emilsson et al. 2008; Illig et al. 2010; Manolio 2010; Schadt 2009). Tailored chemotherapy treatment based on patient (Phillips and Mallal 2010) or tumor (La Thangue and Kerr 2011) genetics is an example of a significant success in applying such discoveries; however, for many diseases, the substantial nongenetic variation in disease or treatment outcomes has limited their utility. Thus, the characterization of the broad set of environmental factors, including those related to chemical exposures, that may contribute to disease is directly relevant to both personalized medicine and environmental health protection (Khoury et al. 2011).

In this review, we explore how next-generation ("NexGen") human health risk assessments of chemicals might take advantage of novel data to better characterize and quantify variability in susceptibility, by using and expanding upon current analytical methods. We begin by describing biological variability through the conceptual framework of the source-to-outcome continuum. Next, the utility of that framework is illustrated in a review of current approaches to describing variability in susceptibility in human health risk assessments. Then, emerging data streams that may be informative in characterizing human variability in susceptibility are described. Finally, we consider the opportunities, challenges, and methods for using emerging data to help assess interindividual variability in responses to environmental chemicals across different decision contexts.

Susceptibility as a Function of the Source-to-Outcome Continuum and Biological Variability

The "source-to-outcome continuum" [U.S. Environmental Protection Agency (EPA) 2007; NRC 2007] is a conceptual framework for human health risk assessment of environmental chemicals in which changes in the sources of chemicals in the environment

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are further propagated within the individual through a series of biological and physiological steps that may ultimately manifest as an adverse health outcome (Figure 1):

- ***Source/media concentrations*** are measures of the chemical, which may change under specific risk management options being considered. A given risk management decision may differentially affect media concentrations depending on local conditions.
- ***External doses*** are measures of exposure (e.g., concentration in air × breathing rate per body weight) to or intake (e.g., amount ingested per body weight) of environmental chemicals, and are related to source/media concentrations by exposure pathways. Sources of variability that may confer susceptibility include differences in behaviors, such as breathing rates, water consumption, and dietary habits (e.g., the amount of fish consumed), and, in an occupational context, use of personal protective equipment.
- ***Internal doses*** are the amounts/concentrations of environmental chemicals or their metabolites at the target site(s) of interaction with biological molecules, and are related to external doses by pharmacokinetic (PK) processes. Susceptibility may arise from differences in compartment sizes and composition (e.g., fat concentration in plasma, which rises during pregnancy) (Roy et al. 1994), as well as differences in the rates of uptake (e.g., fraction absorbed from diet or air), metabolism, elimination, and transport to sites of action (e.g., the blood–brain barrier). Such differences may be due, for example, to genetics (e.g., via polymorphisms in metabolic enzymes, uptake and efflux transporters), other chemical exposures (via metabolic enzyme induction and inhibition), and preexisting health conditions and life stage (e.g., via metabolism and mobilization from tissue storage).
- ***Biological responses*** are measures of biological state (e.g., the concentration of glutathione) altered by interactions with environmental chemicals or their metabolites, and are related to internal doses by pharmacodynamic (PD) processes. Variation leading to differential susceptibility can stem from differences in transport systems, receptors and/or proteins in other toxicity pathways, as well as repair capacity (of, for example, DNA), which in turn are affected by intrinsic and extrinsic factors such as genetics and life stage.
- ***Physiological/health status*** reflects the overall state, structure, or function of the organism and is related to biological responses through systems dynamics, the underlying physiological status of the host to which the chemical-specific perturbation is added. Examples include maintenance and adaptation processes (associated with preexisting health conditions, sex hormone levels, for example), and the accumulation of damage events from past exposures (e.g., loss of alveolar septa from past cigarette smoke

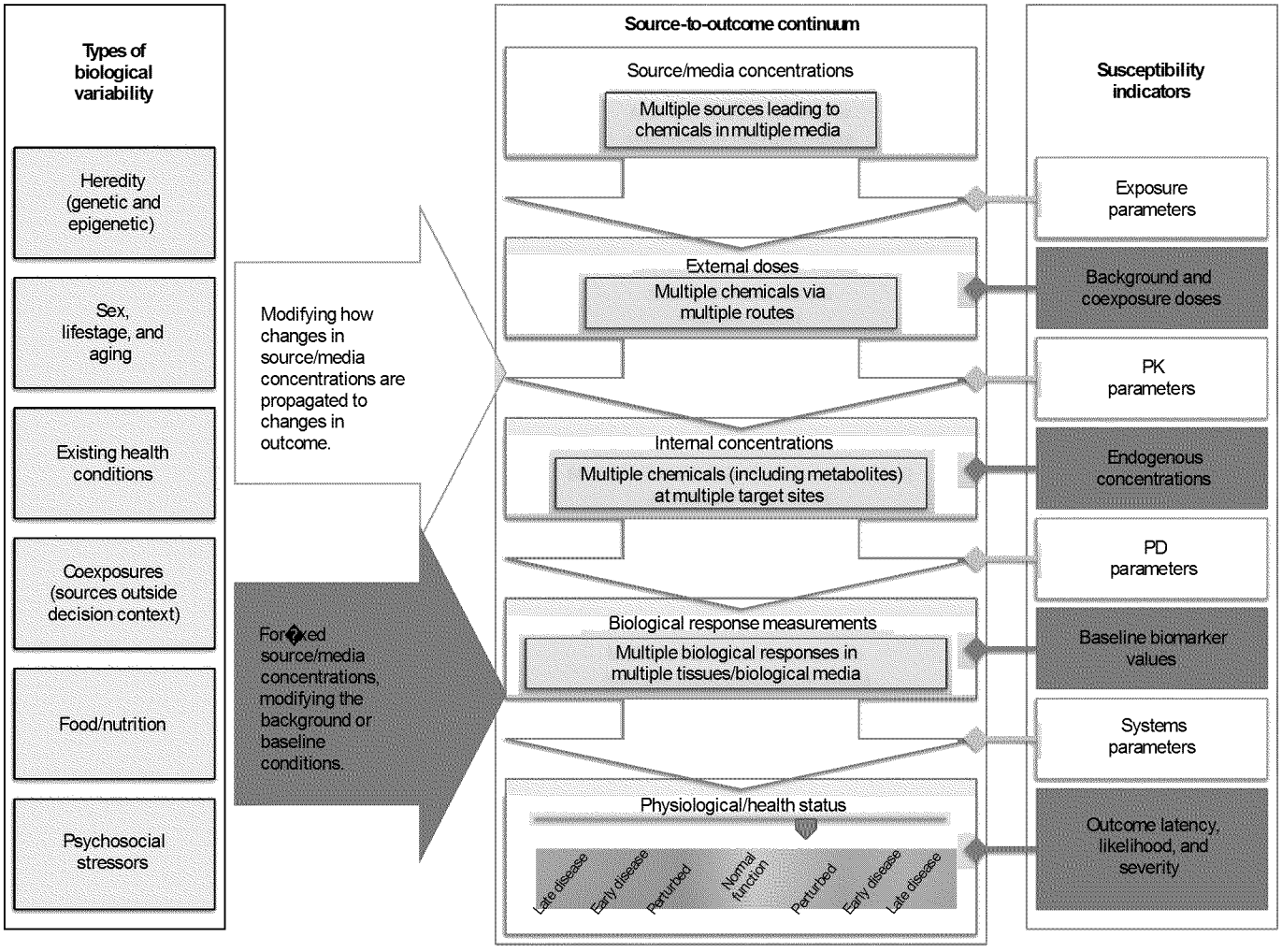

Figure 1. Framework illustrating how susceptibility arises from variability. Multiple types of biological variability intersect with the source-to-outcome continuum, either by modifying how changes to source/media concentrations propagate through to health outcomes or by modifying the baseline conditions along the continuum. The aggregate result of all these modifications is variability in how a risk management decision impacts individual health outcomes. The parameters and initial conditions along the source-to-outcome continuum serve as indicators of differential susceptibility, some of which are more or less influential to the overall outcome (see Figure 2).

exposure). Variation in these can confer susceptibility by altering the likelihood of progression from normal function to mild perturbations, early disease, and late disease. Systems dynamics describes the propagation of biological perturbations regardless of whether they are due to chemical exposure, thus distinguishing it from pharmacodynamics, which describes how chemical exposure causes biological perturbations.

Figure 2 illustrates the distinct effects of different sources of variability on external dose, internal dose, or biological response. The first category of biological variability is indicated by differences in the parameters governing the relationship of one measureable quantity to the next (e.g., external to internal dose, and internal dose to biological response) (Figure 2A,B). In addition, there may be biological variability in the initial conditions for each measureable quantity, as well as the contribution from the source of environmental chemical exposure under consideration for risk management (Figure 2C,D). For example, increases in background exposure to the same or a different chemical(s) may result in saturation of metabolic activation and/or clearance processes, or temporary depletion of cofactors involved in detoxification, such as glutathione, resulting in either attenuation or amplification of the effect of additional increments of chemical exposure on internal dose (Figure 2C). Nonetheless, a biological response with a low background level may be much less altered by additional exposure than one with a high background because of to the cooperativity associated with a relatively higher baseline internal dose (Figure 2D).

Current Approaches to Addressing Variable Susceptibility

Variability for assumed threshold-like dose–response relationships is currently addressed by applying an "uncertainty" or "adjustment" factor (U.S. EPA 2011). The factor to account for interindividual variability in human population has typically been 1, 3, or 10. In some cases, the factor is further divided to separately account for variation in PK and PD (U.S. EPA 2011; International Programme for Chemical Safety 2001). In this context, PD has included both PD and systems dynamics processes described above and in Figure 1. Data permitting, the PK component can be addressed through physiologically based pharmacokinetic (PBPK) modeling, in which case a factor addressing only PD is applied (U.S. EPA 2011). Occasionally, exposure–effect observations are available for particularly susceptible human populations, such as with ozone and persons with asthma (U.S. EPA 2006), or those sensitive to chronic beryllium disease (U.S. EPA 1998), which allows for a data-driven estimation of the likely impact of interindividual variability on human health risk assessments.

For presumed nonthreshold cancer end points, interindividual variability is not currently addressed when risk is estimated from animal studies, with the exception that for mutagenic compounds exposures occurring early in life are weighted more heavily (by a factor of 10 between birth and 2 years of age, and a factor of 3 between 2 and 16 years of age). Cancer risk for susceptible populations, such as smokers who have been exposed to radon, may be calculated in addition to that for a general population (U.S. EPA 2003). Alternatively, adjustments may be made to address susceptible subgroups, such as the sex-specific effects of 1,3-butadiene (U.S. EPA 2002). There have been calls to formally account for variability in cancer dose response (NRC 2009).

Over the past 30 years, several strategies to characterize (predominantly PK) variability combining mathematical models and statistical distributions have developed in parallel. The first strategy, mostly used for data-rich pharmaceuticals, couples empirical PK models and multilevel (random effect) statistical models to extract *a posteriori* estimates of variability from clinical data on patients or volunteers. This "population PK" approach (Beal and Sheiner 1982) seeks to measure variability and to discover its determinants. The second, the "predictive PK," approach takes advantage of the predictive capacity of mechanistic models and assigns *a priori* distributions to their parameters (e.g., blood flows, organ volumes). The parameters having biological meaning can be observed through independent experiments, clinical measurements, or surveillance. Table 1 lists some examples of data sources for developing *a priori* parameter distributions. Monte Carlo simulations are used to propagate the distributions from model parameters to model predictions (Portier and Kaplan 1989; Spear and Bois 1994). A third approach, the "Bayesian PBPK" approach, offers a synthesis of the other two, applying mechanism- or chemical-specific parameter variability data from a variety of independent sources while using population observations of relevant biomarkers of internal exposure and effect to further inform parameter variability (Allen et al. 2007; Bernillon and Bois 2000; Hack 2006). Parameter covariance can be modeled by multivariate prior distributions (Burmaster and Murray 1998) or joint posterior distributions obtained by Bayesian multilevel modeling (Bois et al. 1990; Wakefield 1996). A Bayesian PBPK model-based analysis of the population toxicokinetics of trichloroethylene (TCE) and its metabolites in mice, rats, and humans provides a practical example of how a systematic method of simultaneously estimating model parameters and characterizing their uncertainty and variability can be applied to a large database of studies on a chemical with complex toxicokinetics (Chiu et al. 2009).

PBPK models have been often used to assess variability on the basis of prior parameter distributions obtained from *in vitro* experiments or the physiological literature (Bois et al. 2010; Jamei et al. 2009) and can include genetic information regarding variability. For example, PBPK models can inform the

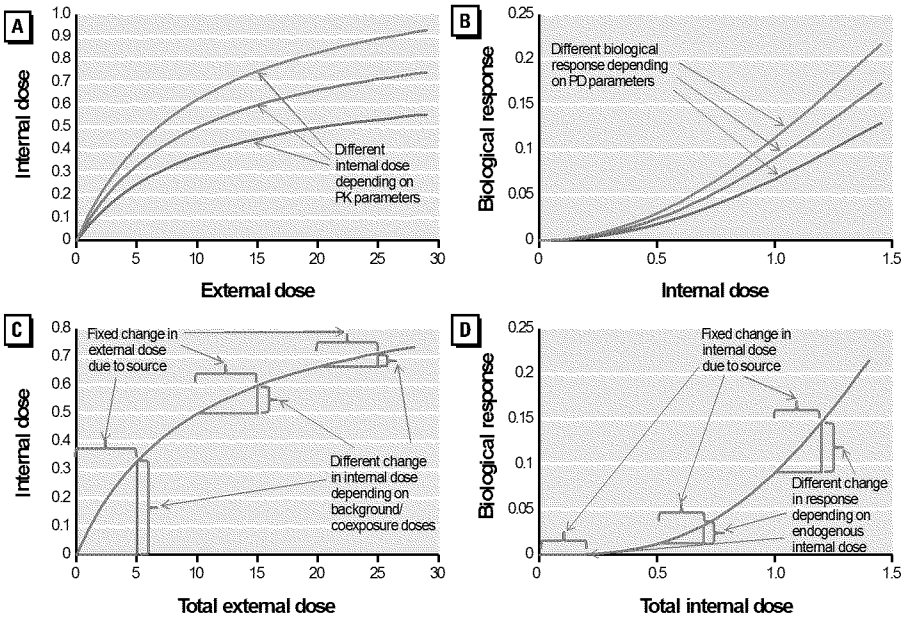

Figure 2. Effects of variability in PK (*A*), PD (*B*), background/coexposures (*C*), and endogenous concentrations (*D*). In (*A*) and (*B*), individuals differ in PK or PD parameters. In (*C*) and (*D*), individuals have different initial baseline conditions (e.g., exposure to sources outside of the risk management decision context; endogenously produced compounds).

implications of polymorphisms in metabolism genes (Johanson et al. 1999). The effects of such polymorphisms on PK of environmental toxicants and drugs have been the subject of many empirical studies (reviewed by Ginsberg et al. 2009c, 2010). These polymorphisms are of particular concern for xenobiotics whose metabolic fate or mechanism(s) of action is controlled by a particular enzyme (Ginsberg et al. 2010), and in such cases genetic variability can profoundly influence enzyme function with implications for internal dose (Figure 1). However, because enzymatic pathways with overlapping or redundant function and other pharmacokinetic factors (e.g., blood flow limitation) can also influence metabolic fate (Kedderis 1997), PBPK models are needed to evaluate the implication of genetic polymorphisms in metabolizing enzymes in human health risk assessment (Ginsberg et al. 2010).

The situation is somewhat different for PD and systems toxicology models. The biologically based dose–response models describe apical or intermediate end point responses as a function of PK-defined internal doses (Crump et al. 2010). However, models designed purely from our understanding of the disease process, such as the role of cytotoxicity and regenerative proliferation in carcinogenesis (Luke et al. 2010b), or the effect of dietary iodide and thyroid hormones on the hypothalamic–pituitary–thyroid axis (McLanahan et al. 2008), require further development to reliably predict an adverse outcome from tissue exposure (the last two arrows in Figure 1), or its variability. Understanding a disease process at the pathway level (i.e., PD and systems dynamics components of the source-to-outcome continuum) is in itself not sufficient to define reliable and informative mechanistic models because of great model sensitivity to uncertain inputs. Most such models are based on equations derived from the classical receptor theory (Csajka and Verotta 2006) and focus on PD rather than system dynamics elements of the disease process and do not attempt to model the full process from tissue exposure to disease outcome.

Emerging Data Streams on Biological Variability

Experimental population-based paradigms to address intrinsic variability in response to exposure comprise multiple levels of biological organization, from molecules to whole bodies. Published examples, reviewed by Rusyn et al. (2010), include animal models and large-scale *in vitro* screening platforms to study population-based genetic determinants. Those studies have also aided in the identification of genetic susceptibility factors that underlie toxicity phenotypes. Complementary to these are genome-wide (Hutter et al. 2012) and exposure-wide (Patel et al. 2010) association studies for assessing human population variability.

Experimental in vitro data on genetic variability. Human cell lines obtained from genetically diverse subjects and multiple populations (Durbin et al. 2010) hold the promise of providing data for assessing genetic determinants of different components of toxic response. Many recent studies have used human lymphoblastoid cell lines, representative of the genetic diversity in populations of European, African, Asian, and North and South American ancestry, to quantify interindividual and interpopulation variability in response to drugs (Welsh et al. 2009). Dozens of studies published in the past 5 years have profiled the cytotoxicity of single to as many as 30 drugs (mostly chemotherapeutics) in hundreds of cell lines. Diverse applications for such a population-based cell model has been suggested. Drug class–specific signatures of cytotoxicity, which could indicate possible shared mechanisms, have been identified and replicated in both cell lines from different populations and for additional compounds (Watson et al. 2011). Furthermore, such studies may potentially inform the prioritization of chemotherapeutic drugs with a sizable genetic response component for future investigation (Peters et al. 2011) and assist in identifying germline predictors of cancer treatment outcomes (Huang et al. 2011).

The utility of such *in vitro* models to toxicology, especially for exploring the extent and nature of genetic components of interindividual variability in PD and systems dynamics, was recently demonstrated (Lock et al. 2012; O'Shea et al. 2011). Quantitative high-throughput screening (qHTS) produced robust and reproducible data on intracellular levels of adenosine triphosphate and caspase-3/7 activity (i.e., biological response) indicative of general cytotoxicity and activation of apoptosis (i.e., physiological status), with utility for variability assessment as follows. First, standardized and high-quality concentration–response profiling, with reproducibility confirmed by comparison with previous experiments, enables prioritization of chemicals based on interindividual variability in cytotoxicity. Second, genome-wide association analysis of cytotoxicity phenotypes allows exploration of the potential genetic determinants of that variability. Finally, the highly significant associations between basal gene expression variability and chemical-induced toxicity suggest plausible mode-of-action hypotheses for follow-up analyses.

Several extensions of these studies can be envisioned to advance the identification of determinants of genetic susceptibility and variability in toxic response. Opportunities include the testing of additional, and more diverse, chemicals (including major metabolites) and concentrations (to account for lower metabolic capacity of these cells). Other specific end points could also be assessed. Further, these studies could be expanded to include larger panels of lymphoblasts and other cell types from genetically and geographically diverse populations. Development of related assay systems to monitor differences in susceptibility to perturbation of communication between cells (e.g., neurotransmission or differentiation signals) could address other aspects of variability not present in cultures comprising only one kind of cell. The development and use of these and other types of *in vitro* assays would be further informed by quantitative comparisons of the PD interindividual variability measured *in vitro* with observable human pharmacodynamics variability *in vivo*. Candidate chemicals for this comparison would be selected environmental toxicants (such as ozone) and pharmaceuticals that have been tested for responses in appreciable numbers of human subjects at different known exposure levels. The extent of interindividual variability in response

Table 1. Examples of data sources for modeling PK and PD variability.

Example	References
Variability in human phase I and phase II metabolism and renal excretion, including in different age groups–neonates, children, and the elderly	Dorne 2010; Ginsberg et al. 2002, 2004; Hattis et al. 2003
Compilations of genetic polymorphisms of specific metabolic enzyme activities:	
Paraoxonase	Ginsberg et al. 2009a
N-Acetyltransferase 1 and 2	Bois et al. 1995; Walker et al. 2009
Glutathione transferases	Ginsberg et al. 2009b
CYP2D6 (cytochrome P450 2D6)	Neafsey et al. 2009b
CYP2E1 (cytochrome P450 2E1)	Neafsey et al. 2009a
ALDH2 (acetaldehyde dehydrogenase 2)	Ginsberg et al. 2009c
Human biomonitoring observations of interindividual differences in biomarkers of exposure (e.g., chemical-protein adducts) or in levels of parent/metabolite	Bois et al. 1996
Variability in physiological parameters for older adults: bodymass, surface area, body mass index, health status	Thompson et al. 2009
Indicators of PD variability	
Human DNA repair enzyme XRCC1	Ginsberg et al. 2011
Human host defense enzymes	Ginsberg et al. 2010
Lung function response to particulate matter	Hattis et al. 2001
Susceptibility to infectious organisms	Hattis 1997

that was observed for different chemicals in *in vitro* assays could also be compared with previously collected sets of *in vivo* human PD variability data (Hattis et al. 2002).

Experimental* in vivo *data. Several proof-of-concept studies that utilized a "mouse model of the human population" have demonstrated the potential for translation to clinical applications and for addressing both PK and PD components of variability (Guo et al. 2006, 2007; Harrill et al. 2009b; Kleeberger et al. 1997; Prows et al. 1997). For example, the extent and nature of TCE metabolism is an important consideration in relating adverse health effects in rodents to humans. Bradford et al. (2011) measured variability in PK for TCE using a panel of inbred mouse strains, revealing marked differences among individual mice (e.g., a greater than 4-fold difference in peak serum concentrations of TCE metabolites). These experimental data on intraspecies differences in TCE metabolism may be used to calibrate the variability in outputs of PBPK models, and thus inform quantitative assessment of variability in TCE metabolism across species.

With regard to PD variability, genetically diverse mouse strains can be used to understand and predict adverse toxicity in heterogeneous human populations. For example, Harrill et al. (2009a) evaluated the role of genetic factors in susceptibility to acetaminophen-induced liver injury in a panel of inbred mouse strains and two cohorts of human volunteers. The authors identified genes associated with differential susceptibility to toxicity in a preclinical phase. This finding has the potential to focus further toxicogenetics research, overcome the challenges of studies in small human cohorts, and shorten the validation period. The data acquired with this model may be used in analyses of individual risk to toxicants. Furthermore, when combined with omics data collected on an exposed population of individual strains, it may be possible to explore underlying genotype-dependent and -independent toxicity pathways involved in PD response (Bradford et al. 2011; Harrill et al. 2009a).

Experiments such as these afford the opportunity to quantitatively understand the interplay between genetics, PD, and systems dynamics. In addition, genetically defined mouse models may be used to supplement the limited data from human studies to not only discover the genetic determinants of susceptibility and understand the molecular underpinnings of toxicity (Harrill et al. 2009a; Koturbash et al. 2011) but also to develop descriptions of variability for use in dose–response and mechanistic evaluation components of human health risk assessments.

Such rodent systems can also be used to assess the role of epigenetics, as well as its potential interplay with the genetic background, in susceptibility. For example, Koturbash et al. (2011) demonstrated that interstrain differences in susceptibility to 1,3-butadiene–induced genotoxicity may be due to strain-specific epigenetic events that are also part of a PD response.

Practical use of this type of experimental information is possible mainly when the mechanistic pathways to human adverse responses are better established. More general application will also depend on the development of suites of rodent models that more fully represent human diversity in both genetics and other factors, such as age (Hamade et al. 2010). Such studies can, in turn, provide important insights concerning the identity and extent of sources of variability that may arise in the source-to-outcome continuum for a given chemical class, physiologic state, or adverse response.

Human clinical and observational data. Genome-wide association studies (GWAS) with disease severity as the phenotypic trait are used to associate genetic loci with risk for complex diseases (Rosenberg et al. 2010). Even though GWAS approaches have uncovered numerous genomic loci that may affect the risk of human disease (Manolio 2010), the identified variants explain only a small proportion of the heritability of most complex diseases (Manolio et al. 2009). Some have suggested that unexplained heritability could be partly due to gene × environment interactions, or complex pathways involving multiple genes and exposures (Schadt and Björkegren 2012).

The GWAS concept is now being applied to identify additional genotype-dependent metabolic phenotypes and to gain insight into nongenetic factors that contribute to the effects of xenobiotics on system dynamics. In animal studies, metabolic phenotype-related quantitative trait loci were shown to be useful in understanding genome × phenotype relationships and how extended genome (microbiome) perturbations may affect disease processes through transgenomic effects (Dumas et al. 2007). In a series of human studies (Gieger et al. 2008; Illig et al. 2010; Suhre et al. 2011), serum collected from two large European cohorts (2,820 individuals in total) was analyzed with nontargeted metabolomics, focusing on endogenous metabolites and covering 60 biochemical pathways. Ratios of metabolites to parent chemical concentrations served as surrogates for enzymatic rate constants. Thirty-seven genes were associated with blood metabolite concentrations and, in some cases, explained a substantial fraction of the variance. Endogenous and xenobiotic metabolites (mostly of drugs) were studied.

Clinical (Brown et al. 2008; Hernandez et al. 2010) and epidemiological (Jia et al. 2011; Wood et al. 2010) studies of acute and chronic effects of ambient air exposures have long had important roles in quantifying human variability in the risks of exposures to widespread toxicants such as ozone and airborne particulates. The addition of GWAS to these established tools has the potential to widen the capability for quantification of effects on susceptibility of many individual genotypic variants that individually have relatively modest effects (Holloway et al. 2012). Establishing the roles of individual pathways in affecting susceptibility via genetic analysis, in turn, has the potential to advance the assessment of effects of other exposures during life that also affect the same pathways. Elucidating these determinants for prominent toxicants, however, requires a very considerable research effort. Nonetheless, this research paradigm provides opportunities to explore variability in adverse responses that is due to physiological states for which *in vitro* and experimental animal models are lacking.

Variability in human response to an agent stems in part from differences in the underlying exposures that contribute to a given disease response prevalence within the population. A person's internal "chemical environment" may be as important for possible disease associations as exposures to the variety of chemicals in the external environment. Under this "exposome" concept (Wild 2005), exposures include environmental agents and internally generated toxicants produced by the gut flora, inflammation, oxidative stress, lipid peroxidation, infections, and other natural biological processes (Rappaport and Smith 2010).

Advances in *in Silico* Methods to Address Human Variability

Modeling of variability is expected to be needed for both data-rich and -sparse chemicals. Recent advances in software, publicly available data and ongoing computational activities in biomedical research should facilitate the development and use of the results of this type of modeling.

Modeling the PK dimension of human variability. Commercial software products [e.g., by Simcyp (http://www.simcyp.com), Bayer Technology (http://www.pksim.com)] are available to explicitly address variability for pharmaceutical or human health risk assessment applications to, for example, adjust dosing for different target patient populations (Jamei et al. 2009; Willmann et al. 2007). Several of these offer generic PBPK models, applicable to "any" substance; however, their substance-specific parameters have to be obtained from *in vitro* experiments (particularly on metabolism) or quantitative structure–property relationships. The variability of subject-specific physiological parameters can be informed by compiled databases (see above) and literature searches (Bois et al. 2010; Ginsberg et al. 2009c), and could include

adjustments or protocols to address limitations in data availability. Quantitative structure–property relationship models or *in vitro* data can also be used to derive substance-specific parameters. These models are being applied in an exploratory fashion in *in vitro*–based assessments (Judson et al. 2011; Rotroff et al. 2010).

Using a Bayesian multilevel population approach, some of the key parameters of these generic models could be calibrated by integrating human observational data with data from lower levels of biological organization. This presents a computational challenge on a chemical-specific basis, because those models are neither particularly parsimonious nor quickly evaluated. Yet an extensive calibration of a complex generic model for a selected number of data-rich environmental or pharmaceutical chemicals could be used as support to develop generic approaches for PK variability treatment in human health risk assessment. For example, generalizations could be made about the extent to which particular enzymes may contribute to overall human PK. Extensions of the approach of Hattis et al. (2002) can also be developed to construct "bottom up" quantitative descriptions of PK variability that can be applied as defaults across classes of chemicals.

Modeling the PD dimension of human variability. Semi-empirical PD models can include observed biomarkers of susceptibility as covariates. Such models are increasingly applied in predictive toxicity and human health risk assessment. Environmental epidemiology also routinely models quantal types of biomarker data in logistic regressions. Harmonizing the tools and models of toxicological risk assessment with those of epidemiological risk assessment, and reconciling their data and results, should facilitate the development of better approaches for background and variability descriptions in NexGen human health risk assessments.

Integrating PK and PD into a systems biology framework. The link between toxicity pathway and "normal cell physiology" models of systems biology could also be further developed and used as the basis to explore potential ranges of human variability. The potential of publicly accessible and curated biomodel and database repositories will be increasingly exploited as familiarity increases in the risk assessment and risk management communities. Importantly, systems biology models can describe background biological processes and the impact of their perturbation and provide a framework for exploring human variability and identifying susceptible populations for targeted assessment and management efforts. Although they come at the price of tremendous complexity, their development can leverage the considerable ongoing effort by the biomedical and pharmaceutical research community to support applications other than toxicant risk evaluation. Further, because of these large-scale efforts, the necessity of sharing and standardization is well understood in the United States. The systems biology markup language (Hucka et al. 2003), for example, is a high-level language developed explicitly to provide a common intermediate format for representing and exchanging systems biology models. Predictive toxicology will benefit from these developments.

The frontier for both PK and PD is in the integration of the rapidly growing information about metabolic networks, receptors, and their regulation with toxicity pathways. The models so far most amenable to quantitative predictions are differential equation models. PBPK models will likely be merged with systems biology and virtual human models. The boundary between PK and PD actually tends to blur as metabolism becomes more and more integrated into detailed models of toxicity pathways when, for example, modeling enzymatic induction by xenobiotics (Bois 2010; Luke et al. 2010a). The variability of the different components of those models will be directly informed by time series of genomic, proteomic, metabolomic data on the chemical species considered. This may provide a framework for assessing the variability in susceptibility to chemically induced effects as influenced by possible metabolic interactions as well as preexisting disease. In time this may facilitate computing the impact of, for example, single nucleotide polymorphisms on the reaction rates of enzymes and receptors and translating these calculations to estimates of human variability (Mortensen and Euling 2011). Ongoing work on simulations of enzymatic reactions or receptor binding at the atomic level (e.g., the potassium channel pore) shows the way forward for predicting fundamental reaction rates by physical chemistry approaches. Prediction of the quantitative impact of sequence or amino-acid variation on the function of the reactive species involved in systems biology models is coming within reach (Giorgino et al. 2010; Sadiq et al. 2010).

Biologically based PD models, such as the systems biology models of response networks (Schuster 2008), models of toxicity pathway perturbations, and biologically based dose–response models proposed to link biochemical responses to apical effects, clearly hold promise (Csajka and Verotta 2006; Jonsson et al. 2007; Nong et al. 2008) but face challenges similar to those that hampered the use of biologically based cancer models (Bois and Compton-Quintana 1992; Chiu et al. 2010). To explore the extent of human variability in response to toxicant and stressor exposures, the various steps in the relevant causal path need to be modeled quantitatively and on a population basis. A problem is that the quantitative linking of omics biomarkers to risk is missing. For many markers (e.g., of apoptosis, cell division), the linkage to risk is highly uncertain (Woodruff et al. 2008), so the ranges of possible variability may be very large. Further, the ability to reinforce information by linking with the impact of injury on multiple targets is also limited because such links are generally not well understood.

Implications for NexGen Human Health Risk Assessments

Multiple "tiers" of human health risk assessment needs, requiring different levels of precision, can be envisioned. These include screening-level analyses of multiple chemicals to inform the prioritization of management and enforcement actions across communities, ensuring protection across the population to widespread exposure to legacy contaminants, or identifying subpopulations for which differing risk management options might be applied.

In the lowest (simplest) tier of assessments, evaluations are expected to primarily rely on the results of high- and medium-throughput *in vitro* screening tests in mostly human cell lines, as well as complementary *in silico* predictive methods. The Tox21 collaboration (Collins et al. 2008) is leading the field in exploring how a broad spectrum of *in vitro* assays, many in qHTS format, can be used to screen thousands of environmental chemicals for their potential to disturb biological pathways that may result in human disease (Xia et al. 2008). Such data on toxicologically relevant *in vitro* end points can be used as toxicity-based triggers to assist in decision making (Reif et al. 2010), as predictive surrogates for *in vivo* toxicity (Martin et al. 2010; Zhu et al. 2008), to generate testable hypotheses on the mechanisms of toxicity (Xia et al. 2009), and to develop screening assays based on pathway perturbations. The extent of interindividual variability in toxic response to be estimated from these types of assays can be informed by empirical data and PK/PD models that address multiple factors in the source-to-outcome continuum as described in Figure 1. The genomic component of variability may be partially informed by test data from genetically diverse but well-defined human cell lines, such as from the HapMap (http://hapmap.ncbi.nlm.nih.gov/) and 1000 Genomes (http://www.1000genomes.org/) projects. For example, emerging data based on standardized and high-quality concentration–response profiling can help inform characterizations of the extent of interindividual variability in cytotoxicity. When chemical-specific estimates are lacking, the range of interindividual variability for structurally related compounds may be informative, in a read-across approach. Quantitative data characterizing the range in response (e.g., size and variance) may be integrated with probabilistic default distributions addressing the remaining key sources of interindividual

variability. Quantitative estimates of PK variability would be also incorporated. In addition, factors such as life stage and background exposures may be particularly important considerations for approaches accounting for baseline differences in the spectrum of the "chemical environment" (Rappaport and Smith 2010), in interpreting results from the omics assays, and in evaluating the potential contributions of nongenetic variability factors.

At these lower tiers, a probability distribution may best acknowledge the many uncertainties involved in making inferences with limited data. Systematic analyses of chemical sets will be needed to refine distributions for the chemical-specific and general case. For instance, external comparisons of *in vitro* measures based on genetic variability in pharmacodynamics to *in vivo* observations may inform the choice of distribution used for a particular chemical or chemical category. Standard categories, comprising different size and variance distributions for multiple variability factors that can then be applied to other chemicals, may emerge from these analyses. The ranking and grouping of chemicals for the application of these distributions may be based on structural class, the relative extent of observed variability, a common determinant of variability (e.g., as identified in GWAS analysis of cytotoxicity phenotypes), or other factors (e.g., likelihood of coexposures or confounders). Compounds demonstrated or predicted to have highly variable toxic responses may also be given a higher priority for further study, in combination with chemical and other expected modifiers of susceptibility.

At higher tiers of NexGen human health risk assessments, animal and in some cases human data are available for evaluating dose–response relationships, major pathways for some of the critical toxicities for risk assessment can be reasonably well understood, and some *in vivo* human data relevant to those pathways may be available. For some chemicals, sensitive populations may have been identified and studied using omics technologies. In the case of ozone, for example, gene expression data and genomic markers may be collected on individuals of high and average sensitivity. Toxicity pathways exhibited in cultured airway epithelial cells exposed to ozone may also be compared with those in humans exposed *in vivo* to ozone. Such data will aid a better characterization of the dose–time–response severity relationships at low doses. In other cases, where individuals are studied epidemiologically, the current bioinformatics analyses lack power and require pooling of subjects to detect trends, losing variability estimation in the process. In such cases, there will be a need to couple default descriptions of PD variability with PBPK modeling to obtain an overall prediction of variability. In the future, new hypothesis-based molecular clinical and epidemiological approaches that integrate emerging biological knowledge of pathways with observations of physiological disease status, markers of early biological response, and genetics are likely to provide the way forward with population-based descriptions of variability.

Conclusions

Emerging data streams can inform multiple aspects of biological variability, be used in different modeling approaches addressing PK and/or PD variability, and have application across different chemical screening and evaluation schemes. Successful examples of addressing PK variability include the development and application of a Bayesian PBPK model–based analysis systematically estimating model parameters and characterizing their uncertainty and variability for TCE, a chemical with complex toxicokinetics (Chiu et al. 2009). Additionally, data from animal models and large-scale *in vitro* screening platforms that have incorporated population-based genetic determinants (reviewed by Rusyn et al. 2010), have provided insight into the extent of genetic variability in response to a diversity of toxicants, as well as aided in the identification of genetic susceptibility factors that underscore the development of toxic phenotypes. Hypothesis-based molecular clinical and epidemiological approaches to integrating genetics, molecular pathway data, and clinical observations and biomarkers are likely to contribute to population-based descriptions of variability. Complementary to these are genome-wide (Hutter et al. 2012) and exposure-wide (Patel et al. 2010) approaches for assessing human population variability in toxic response. Opportunities exist to employ these emerging data streams in the development of *in silico* predictive models for application in a range of decision-making contexts.

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EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

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The Biology of You

–by National Research Council staff

What makes you, you? From a biologic perspective, a common answer is, your genes. The answer seems simple enough—or is it? Certainly, many of our traits are coded in our genome and passed down from parent to child. But as scientists explore questions about why people differ—what makes us healthy, and what makes some of us susceptible to developing a disease—evidence suggests that there is more involved than just "your genes." Variation in human populations is enormous, so either the possible genetic sequences are indefinite or perhaps there is more to "you" than genetics alone.

Understanding human variability is important in medical and public-health communities. Discussions about the appropriate public exposure limits for environmental pollutants or the effectiveness of vaccines and medical treatments can be better informed with improved insight into who is and how many are at risk because of biologic differences. Consequently, some scientists argue that more research on human variability is both practical and urgent.

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Biologic Factors That Underlie Individual Susceptibility

–by Kellyn Betts, edited by National Research Council staff

On April 18–19, 2012, the National Academy of Sciences Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH) hosted a public meeting on the state of the science regarding biologic factors that govern how people vary in their responses to environmental exposures. A 2010 National Research Council report, *Science and Decisions: Advancing Risk Assessment,* noted that it is difficult to estimate average population risk without understanding individual risks. In other words, to address population susceptibility to environmental stressors, it is critical to address individual variability. Thanks to emerging molecular techniques, scientists are gaining a new understanding of inherent differences among people. That information can be used to predict how people will differ in their susceptibility to environmental stressors and to inform risk-assessment and public-health practitioners who are tasked with protecting vulnerable populations.

Why does one person fall ill after exposure to a particular environmental stressor and another remain unharmed? Variability, the true differences in people's attributes, holds the answer. Variability can be

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> ***Understanding individual variability is central to understanding susceptibility, identifying vulnerable populations, and understanding mechanisms so that we can identify and develop methods to intervene for the most vulnerable. It may lead to novel treatments and public-health interventions for environmental health problems.***
>
> **—John Balbus**

IN THIS ISSUE

This newsletter and additional information about the committee and its activities can be found at http://nas-sites.org/emergingscience/ . The newsletter is prepared by National Research Council staff to keep you informed of activities of the Standing Committee on Emerging Science for Environmental Health Decisions. The views expressed in the newsletter are those of the meeting presenters and participants. The newsletter does not represent either formal consensus conclusions of the attendees or positions necessarily endorsed by the National Research Council.

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caused by external factors, such as duration of exposure to a pollutant or a person's workplace environment. Endogenous biologic factors, such as genetics and pre-existing illness, are also sources of variability, William Farland, of Colorado State University, explained. Understanding variability is extremely important because "variability is inherent in populations," he emphasized; it's not going to disappear. John Balbus, of the National Institute of Environmental Health Sciences (NIEHS), noted that the ability to characterize variability at the individual level in human and laboratory animals is essential for the protection of human health and understanding variability is therefore the second goal of the newly released NIEHS 5-year strategic plan. Previous ESEH forums have addressed tools and technologies for characterizing exposure; the current meeting would focus on new methods and insights to help to characterize individual biologic variability, Farland said.

How much variability is there in human populations? Meeting participants described a number of endogenous sources of variability. Nathaniel Rothman, a senior investigator at the National Cancer Institute, discussed the scope of human genetic variation and described how genetic variations may contribute to disease. The amount of variability in humans "is striking," Rothman said. Genetic variance between people ranges from such very small differences as single-nucleotide polymorphisms (SNPs; variations in which a single nucleotide in the genome sequence is altered) to such very large differences as chromosomal rearrangements. It is estimated that there are about 10–12 million common SNPs, which have more than a 10% minor allele frequency (the ratio, in a population, of the number of chromosomes that

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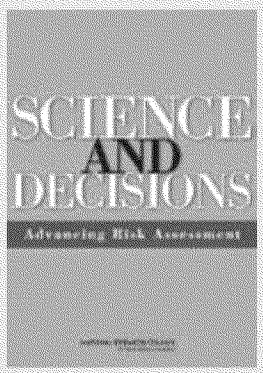
Variability, Susceptibility, and Vulnerability

Variability—the true difference in attributes due to heterogeneity or diversity. Variability is usually not reducible by further measurement or study, although it can be better characterized.



Susceptibility— the capacity to be affected. Variation in risk reflects susceptibility. An individual can be at greater or less risk relative to the an individual in the population who is at median risk because of such characteristics as age, sex, genetic attributes, socioeconomic status, prior exposure to harmful agents, and stress.

Vulnerability—the intrinsic predisposition of an exposure element (person, community, population, or ecological entity) to suffer harm from external stresses and perturbations. Vulnerability is based on variations in disease susceptibility, psychological and social factors, exposures, and adaptive measures to anticipate and reduce future harm, and to recover from an insult.

To set the stage, Farland and other meeting participants referenced the 2012 National Research Council report **Science and Decisions: Advancing Risk Assessment,** *which provides practical scientific and technical recommendations for improving risk assessment, including the definitions given above.*

BIOLOGY OF YOU, *cont. from page 1*

The Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH) has explored many of the facets of human variability. This newsletter focuses on emerging science and approaches to identification and characterization of biologic variability in humans. ESEH meetings have focused on epigenetics, the microbiome, and how environmental exposures influence these aspects of human biology. Recently, the ESEH committee delved into genomic plasticity and the non-DNA elements of the genome that enable humans to adapt to environmental changes. The meetings have made it clear that the biology of what makes us individuals is complex.

2012 marks the fifth year of ESEH meetings that explore the new science of the human genome, epigenome, microbiome, and other biologic factors and how they interact with our environment. So, what makes you, you? The answer is not simple. Please join us in 2013 as we continue to explore the scientific advances that can help us to answer this question and the implications of the new science for environmental health decisions.

INDIVIDUAL, *cont. from page 2*
carry a less common gene variant to the number that carry the more common variant); there may be 30–50 million uncommon SNPs, which have minor allele frequencies of 1–10%; and it is possible that there are more than 100 million rare SNPs, with frequencies down to 1%. In other words, variation is inherent in our genome.

Claudia Miller, of the University of Texas at San Antonio, emphasized the need to consider genetics and human exposure to environmental chemicals in the context of evolution. Most chemical pollutants are "novel substances" that were developed after World War II, Miller said. We must ask, What is the variability in human ability to metabolize and excrete these substances that were so recently introduced into our environment?

> ***There are so many SNP variants that some people wonder whether there might be variation in almost every one of the 3.1 billion base pairs in the human genome.***
>
> **—Nathanial Rothman**

Emerging Technologies

Current methods for detecting genomic variability have focused mainly on DNA, such as the use of off-the-shelf chip technologies, candidate genes, and the newer "agnostic scans" that are possible with genomewide association studies (GWASs). The technologies for detecting DNA variance span molecular genetic methods for sensing smaller differences and cytogenetic methods for detecting larger differences, Rothman said. Today, off-the-shelf chip technologies provided by such companies as Luminol and Affymetrix are capable of interrogating about 10% of the most common SNPs. Rothman stressed that there is a "tremendous amount of genetic variation that so far has not been analyzed in association and genetic epidemiology studies." However, GWASs are enabling scientists to better discover links between genetic polymorphisms and obesity and diseases, including hepatic cancer, chronic leuokocytic leukemia, prostatic cancer, diabetes, and coronary arterial disease. Rothman emphasized that he expects an "explosion in the number of new genetic findings" as technologies for interrogating uncommon SNPs become available.

Rothman cautioned that genetic studies should not be conducted in isolation from other factors that contribute to variability. Integrating all factors that contribute to variability into the same study has the potential to provide mechanistic insight, clarify dose–response relationships, and make it possible to evaluate low-level risks more effectively. For example, Rothman and colleagues recently discovered that overlaying multiple risk factors for bladder cancer allowed them to differentiate risk subgroups. They developed weighted "gene scores" based on SNPs known to be associated with bladder cancer. The gene scores allowed Rothman and colleagues to sort people into quartiles of low, medium, and high genetic risk for bladder cancer. They applied the gene scores to male 50-year-old never, former, and current smokers. Whereas absolute risk for male 50-year-old current smokers is 6.2%, Rothman's method estimated a 9.9% risk for current smokers in the high-genetic-risk subgroup as determined by the gene score. In public- health terms, "eliminating smoking in 100,000 people who have the highest genetic risk could eliminate 8,000 cases of bladder cancer," Rothman said. Rothman hopes that the gene-score approach in his bladder-cancer research will serve as a model for looking at genetic and environmental factors involved in other diseases, but he noted that the methods used in the bladder-cancer research first need to be

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Sources of Biologic Variability

- Sex
- Genetics and epigenetics
- Health status (new and pre-existing health conditions)
- Life stage and aging
- Microbiome

Rothman, Farland, and other meeting participants described some of the biologically based factors that contribute to human variability and thus population heterogeneity. Much of the current research is focused on characterizing the sources of variability such as those listed above and their interplay with human behavior and environmental factors that give rise to a person's disease susceptibility.

INDIVIDUAL, *cont. from page 3*
replicated. Rothman added that integration may also identify new environmental health hazards and ultimately help researchers to develop more effective prevention, screening, and treatment strategies.

Scientists are also beginning to use cutting-edge technologies that go beyond DNA—including technologies that involve RNA, proteins, and metabolites—to explore other dimensions of the biologic variability of living organisms. Eric Schadt, of the Mount Sinai School of Medicine, has been focusing on identifying tools for investigating how perturbations affect living systems by looking beyond DNA. Pacific Biosciences has created what Schadt terms a super high-resolution microscope, officially known as Single Molecule Real-Time, or SMRT®, that capitalizes on recent advances in nanotechnology, molecular biology, and optics. The instrument enables researchers to observe the activity of single molecules, such as DNA or RNA polymerases, in real time, Schadt explained. The aim of using such technology is to get a better handle on the complexity of living systems to identify changes within and differences between individuals that may be caused by external perturbations. "Once we can understand the networks, we can move away from a one-dimensional single-gene view and look at how changes in one part of the system give rise to changes in other parts of the system.," Schadt said. The technology also enables researchers to look beyond internal molecular states and microenvironments to look at, for example, how a person's microbiome or the microbiota that the person encounters in the environment interacts with his or her DNA.

> ***We want to start modeling how perturbations, whether genetically or environmentally induced, are being propagated across the system.***
>
> **—Eric Schadt**

Schadt also explained how the technology has direct use in connection with public health. For example, in a single day, SMRT was able to sequence the *E. coli* strain from a 2011 virulent outbreak in Germany and compare it with strains collected from around the globe. The results, published in the *New England Journal of Medicine* last year, showed definitively that the virulent *E. coli* were enteroaggregative, not enterohemorrhagic as other researchers had suspected. Schadt's research group also discovered that the German outbreak strain acquired plasmids—including a shiga toxin gene—that caused greater virulence than other *E. coli* strains. The inserted viral genes caused epigenetic changes throughout the *E. coli* genome and as a result increased virulence in the host, Schadt explained. In short, the SMRT technology enabled Schadt and his colleagues to see where a bacterial virus punched into the bacterium and added its own genome and how the viral genome integrated into the host system. The researchers also found that the German outbreak strain exhibited increased antibiotic resistance because of horizontal gene transfer with enterohemorrhagic strains.

SMRT may be useful for real-time pathogen monitoring. In a pilot study, Schadt and colleagues analyzed sewage samples from a community in California. They were able to detect respiratory viruses and loosely correlate the increasing load of influenza virus in sewage with an influenza outbreak. They were also able to detect pathogens that are commonly associated with foods, such as peppers, tomatoes, and chicken. On the basis of that information, they could roughly estimate the dietary intake of the community, and this could be useful for characterizing nutritional differences between different populations in molecular-epidemiology studies, Schadt said. Such information is "directly actionable," he argued. Real-time pathogen monitoring not only facilitates outbreak detection but could provide information about environmental conditions, such as nutrition, that could serve as the basis of public-health interventions or other decisions.

Testing for Variability

In vitro screening (cell-based or tissue-based assays) can fill in important gaps in 21st century toxicity testing related to individual variability, said Fred Wright, of the University of North Carolina at Chapel Hill. In vitro screening with human cells can be particularly useful in heritability analyses, identification of mechanisms that might underlie variability via genetic mapping, and characterization of average responses and variations among chemicals for priority-setting. Many of the principles established through in vitro work with pharmacogenomics, particularly cytotoxicity screening

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of anticancer agents, can be applied to the testing of chemical agents, he said. Screening of many human cell lines can unmask sources of heterogeneity that would otherwise be hidden. Chemicals that vary in their effects in the population may need to be ranked for further testing by using additional in vitro or in vivo approaches.

Harvey Clewell, of the Hamner Institutes for Health Science, cautioned that scientists must take care in the choice of cells to be used in vitro studies. He conducted a literature search of arsenic exposure and genomics that revealed that immortalized cell lines yielded results similar to those with primary cells, but tumor-derived cell lines did not.

In vitro systems yield only a partial view of variability and susceptibility to chemical hazards, noted Weihsueh Chiu, of the US Environmental Protection Agency (EPA). However, variability at the molecular, cellular, and tissue levels is integrated in animal and epidemiologic studies that examine whole organisms and populations, respectively. The integration can be probed by using measures of dose and effect biomarkers and clinical outcomes, which provide systemic linkages between exposure and tissue dose (pharmacokinetics); between tissue dose and systemic response, such as a change in hormone concentrations (pharmacodynamics); and finally between systemic response and the likelihood of a disease outcome. By linking to clinical outcomes, Chiu said, animal and population studies can incorporate integrated information on baseline risk and susceptibility, including variability in an organism's robustness in the face of perturbations and its ability to return to homeostasis after a challenge.

Animal and epidemiologic testing has some drawbacks in assessing individual variability, given that, as many meeting attendees commented, both the *dose* and the *host* determine whether an exposure acts as a poison. Animal studies have been handicapped in their ability to assess individual variability by their general use of a single strain of one or two species or an out bred stock, said John. E. French, of the National Toxicology Program. In addition, studies to evaluate the effects of chemical exposures typically are conducted only on young healthy members of inbred animal strains that have little genetic diversity, said Joel Schwartz, of Harvard University.

However, French proposed a laboratory-mouse resource, the Jackson Diversity Outbred (J:DO) stock available through Jackson Laboratories, that could be used to improve the assessment of individual variability and to develop population-based models for environmental exposures, toxicity, and disease. The J:DO mouse was created by Gary Churchill and colleagues from the Collaborative Cross stock, an advanced recombinant intercrossed line developed over the last decade by mouse geneticists led by David Threadgill, of North Carolina State University. The Collaborative Cross stock was created by random outcrossing of eight unique and genetically diverse inbred mouse strains: five laboratory-derived and three wild-derived. When the genetic diversity of the first Collaborative Cross inbred lines were developed and assessed, researchers

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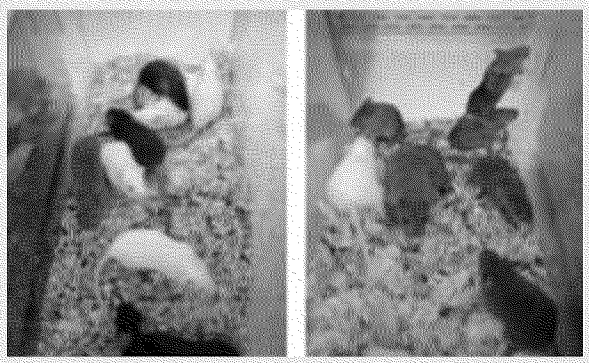
The J:DO Mouse



Siblings from randomly bred J:DO mice created from the Collaborative Cross random 8-way outcross. *(Images courtesy of Dr. Karen Svenson, The Jackson Laboratory)*

French described some of the features of the J:DO mice that make them very useful for determining the wide range of variability and response to toxic exposure. The J:DO mice have obvious phenotypic differences, like size and coat color, representative of their genetic diversity. Every mouse also has either equal to or greater than 10% minor allele frequency. This helps illuminate the consequence of rare allele variants that occur very frequently, French said.

INDIVIDUAL, *cont. from page 5*
were able to observe over 45 million segregating SNPs—a number similar to that in humans, French said.

The J:DO stock's founding population was created from random outcross mating of 144 pre–Collaborative Cross male and female mice. In contrast, most outbred stocks used in toxicology have small founding populations—no more than two or three males or females each—and thus "limited genetic diversity," French emphasized. French and colleagues are testing the J:DO mouse's ability to represent individual variability in response to exposure to benzene. Their findings suggest that the mice can function as a tool to help scientists to analyze and define the range of variations in susceptibility or resistance to toxicity and disease. Their work also shows that the mice can aid in identifying candidate genes and regulatory sequences of causal mechanisms and functional validation through hypothesis-based research testing.

Schwartz outlined how epidemiology studies are useful for looking at sources of variability and susceptibility. For example, epidemiology studies have demonstrated that the association between bone lead and heart-rate variability is pronounced in patients who have metabolic syndrome. They have also demonstrated that air pollution is associated with many health outcomes that are common in people who have diabetes. The collection of such studies indicates that diabetes may be an important modifying risk factor in the effects of air pollution or lead exposure.

Schwartz emphasized that humans obviously have much more diversity in age, health status, genetics, and environmental exposures than is captured by classical animal toxicology studies. Consequently, if there is a threshold dose (such as a no-observed-effect level) of a particular toxin in humans, "we expect it to vary" from one person to another, he said. Classical toxicology studies often identify a threshold below which exposure to a given substance does not cause harm. Although individual people may have thresholds, a growing body of evidence suggests that such thresholds may not exist for the human population as a whole, Schwartz said. He argued that the conglomerate of variability factors in human populations (and hence in epidemiology studies) suggests a linear no-threshold response to environmental exposures at low doses. As an example, he pointed out that the latest research on lead and cognitive effects suggests that there is no threshold. Similarly, a followup that he conducted of the Harvard Six Cities study, which linked excess mortality to exposure to increasing concentrations of particulate air pollutants, showed that the effect of exposure to particles is linear down to extremely low concentrations, approaching the background concentrations. Farland commented that "the impact of individual thresholds on population distributions of response is something that we really need to look at."

> ***If we can identify the sources of variability and the distribution of susceptibility, we can provide important information for decision-making.***
>
> **—Joel Schwartz**

Schwartz pointed out that evaluating geographic distributions of risk and of incremental increases in risk would be extremely valuable. Such a strategy would allow researchers to evaluate both socioeconomic and biologic factors that modulate risk and could be an important tool for planning interventions and improving public health. He also argued that regulators need to start thinking about how to use epidemiology studies in the exposure range of interest in setting standards and about how to use information from the studies in identifying sources of variability.

"The challenge is to integrate different levels of biologic organization when you have different data streams that are interrogating different levels," Chiu said. As you move from the molecular level to the level of the whole organism—that is, to greater levels of biologic organization—more and more sources of variability come into play, he explained. Testing with molecular biochemical assays can identify variability in the rates of reaction in situations in which people who have different genetic backgrounds have enzyme affinities that differ slightly. In a cell-based assay you can also detect differences in the intracellular network that is responding to a given chemical concentration, which can be used to generate some sort of bioactivity measure. However, variability in one enzyme is integrated into systems and networks as you move up to the whole-organisms

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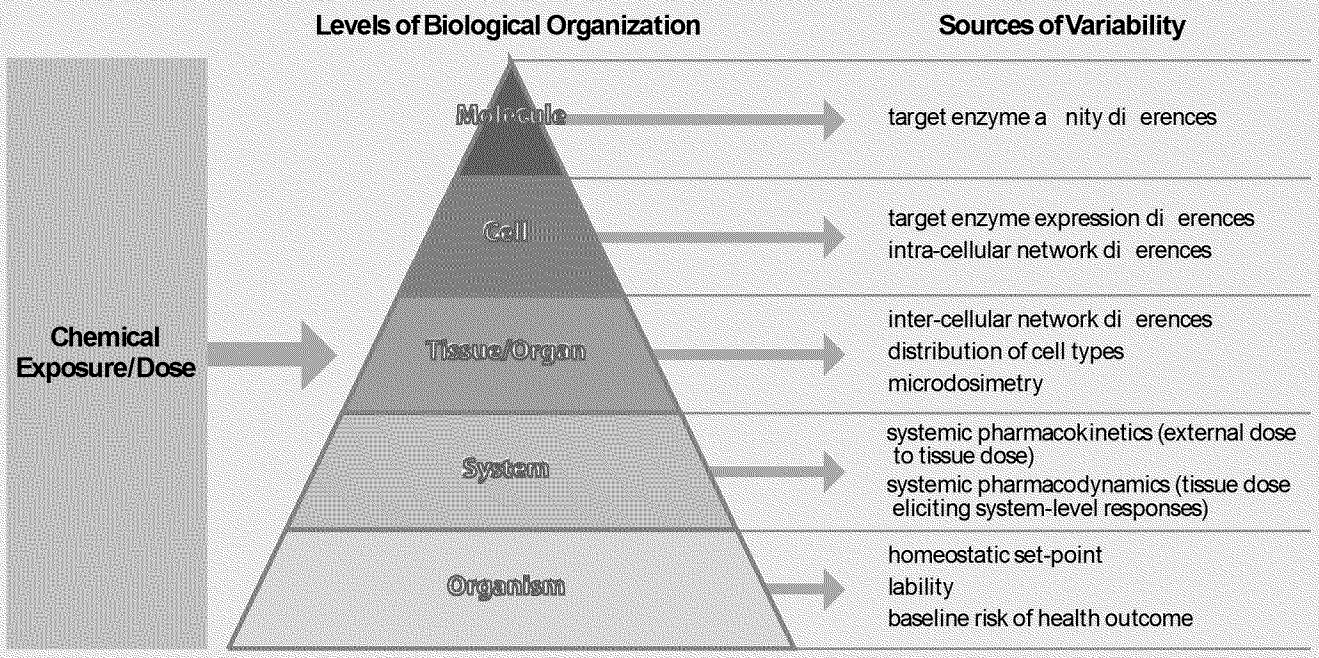
Variability as a Function of Biological Organization



Chiu noted that research on human variability occurs across different levels of biological organization. At each level there is an internal chemical concentration, or dose, that elicits responses (i.e. variability outcome) based upon such biological factors as genetics, health status, and life-stage. Chiu emphasized that data at higher levels of biological organization recruit more sources of variability.

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level. "That integration may amplify or dampen individual sources of variability," Chiu cautioned. Tools to model how sources of variability propagate through the system would help scientists to integrate the available data, he said.

Variability Informing Risk Assessment

"Risk assessment is preventive medicine," said Mike Dourson, of Toxicology Excellence for Risk Assessment. If done appropriately, risk assessment prevents disease and reduces the workload of clinicians, he explained. In a typical assessment, risk assessors pinpoint a critical effect of a pollutant exposure, defined as the first adverse event or its known and immediate precursor that occurs as the dose increases. Risk assessors try to determine the most likely outcome in sensitive groups—not individuals—often on the basis of data on experimental animals or another group of humans, Dourson said. However, the available data that can be used for risk assessments are often insufficient. Consequently, EPA, the Food and Drug Agency (FDA), and other risk assessors use defined uncertainty factors (also called safety factors) when toxicity data or other data are unavailable. Uncertainty is typically addressed by dividing a risk calculation, such as a no-observed-adverse-effect level (NOAEL) of the critical effect, by a default uncertainty factor of 10, which is generally considered to be conservative. "The practice of dividing the NOAEL by 10 implies population variability greater than [a factor of] 10," Dourson said. However, the degree to which uncertainty factors are overprotective or insufficient is usually unknown.

Duncan Thomas, of the University of California Los Angeles, reasoned that uncertainty about population variability has two dominant sources: imperfect knowledge about biologic systems and fundamental randomness in biologic systems. He described mathematical methods for quantifying uncertainty that are based in part on direct biologic measurements. However, he said, the greatest challenge is in dealing with "the unknown unknowns," the factors that contribute to heterogeneity that we do not know about and therefore cannot measure.

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SCIENTIFICALLY SPEAKING

Lauren Zeise is the deputy director for scientific affairs in the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. She is also a member of the Standing Committee on Emerging Science for and contributed greatly to the planning of the meeting on biologic variability. Her research focuses on human individual variability, dose–response relationships, uncertainty, and risk. She shared her views on biologic variability, environmental health, and research looking forward.

Lauren Zeise

Q. Why study biologic variability?

A. Protecting the public's health from exposure to environmental chemicals cannot be accomplished without explicit consideration of biologic variability. Government agencies, medical professionals, and businesses all make assumptions about biologic variability in their decision-making that affects intentional and collateral human exposures. The assumptions are often based on understanding developed in the 1980s of how much and why people respond differently. Emerging molecular and apical evidence from epidemiology, in vivo and in vitro toxicology, and systems biology is providing newer understanding and reasons to reassess current approaches. It is also showing the way to more targeted interventions both for medical decision-making at the individual level and for environmental decision-making for communities and other groups with regard to age, pre-existing health conditions, economic disadvantage, and other factors.

Q. Did you gain any surprising ideas or insights from the meeting on biologic variability?

A. First, I think that the meeting deepened my appreciation of the broad range of tools that can be brought to bear to understand variability. Single-nucleotide polymorphisms (SNPs) and genome-wide association studies have provided insight into genetic variability and how it may be related to susceptibility, as Nat Rothman discussed. But the meeting also highlighted some profound limitations of current tools that can be quite crude in identifying important genetic and epigenetic modifications that can affect disease states. Eric Schadt's talk reminded us through a case example that SNP analyses led the medical community astray in understanding gene targets for leukemia therapy. The silver lining was that the mistaken inference was corrected by using a powerful new approach that enabled the analysis of larger sequences and gene relationships. The meeting also discussed tools for examining variability due to epigenetic differences, but they clearly are limited in the scope, circumstances, and transience of epigenetic changes that they can examine. Second, at the population and subgroup levels, molecular biology is enabling improved inferences regarding dose–response relationships and sensitive groups. Joel Schwartz and Nat Rothman provided striking examples of reduced and enhanced susceptibility associated with genetic polymorphisms that code for activation and detoxification enzymes, behavior, and other nongenetic factors.

Q. Considering the importance of exposure variability in environmental health, is focusing research on biologic variability putting the cart before the horse?

A. Exposure variability clearly is important. Biomonitoring, -omics methods, and new environmental monitoring tools to improve understanding of individual exposures, environmental-exposure hot spots, and variation clearly hold promise and have been discussed in previous meetings. Biologic variability drives population risk that occurs from exposure, so to protect public health and target interventions wisely we need to pay attention to it. In addition to genetic factors, Joel Schwartz showed substantial variability in response seen epidemiologically due to socioeconomic factors and how one might use this and other information to model risk in susceptible populations. His theoretical simulation demonstrated, using reasonable assumptions, that some sensitive groups may face an inordinately high risk of heart attack do to exposure to particulate matter—greater than a 20% absolute risk—whereas the

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Thomas demonstrated mathematically how an "unknown" genetic risk factor or risk modifier could contribute substantially to population heterogeneity without being accounted for by default uncertainty factors in risk assessment. He argued that the use of default uncertainty factors is inadequate to regulate "residual genetic heterogeneity" but acknowledged that risk assessors might be able to use GWAS-based heritability estimates to inform their efforts.

Human variability data may be able to reduce uncertainty in risk-assessment calculations, Chiu argued. In most cases, risk assessment begins with animal toxicology data. Risk assessors use modeling and other techniques to quantify a benchmark dose for a point of departure based on animal dose–response data, he explained. The next step is to derive a human equivalent dose from the benchmark dose. That can be done through various empirical approaches, such as dividing by an uncertainty factor or allometric scaling (a method of accounting for differences in body size), physiologically based pharmacokinetic

> ***I have great difficulty with the idea of using arbitrary safety factors of 10 to pretend that we are protecting the most sensitive individuals in the population.***
>
> **—Duncan Thomas**

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majority of the population faces a considerably lower risk. That raises equity as an additional concern for public-health intervention and further motivates us to understand the basis of biologic variability in assessing environmental-health strategies.

Q. Will a shift in focus from population variability to individual variability require a change in risk-assessment paradigms?

A. An appreciation of individual variability can affect risk assessment in various ways. Stakeholders and decision-makers who gain an understanding of large susceptibility differences will call for the groups at greater risk to be identified and ask for some appreciation of quantitative differences. A number of environmental regulations require that susceptible groups be addressed in mitigation and standard-setting. Second, the average risk, or "population risk," is driven by the array of individual risks, so understanding of the risks in the susceptible groups provides a better basis for calculating population risk. Joel Schwartz illustrated how a variety of factors can increase risk; some groups face heightened risk, and others face very high risk. The people at increased risk are captured in the right "tail" of the risk distribution. Risk in the "median" person can then be a lot lower than the population risk. People in the right tail are targets for intervention. Finally, for common health conditions, such as asthma and cardiovascular disease, that are affected by environmental toxicants, a better understanding of biologic variability leads to better descriptions of the dose–response relationship at low environmental levels and of the need to depart from the assumption of a population threshold (below which no harm is expected). Those dose–response relationships can be used in economic assessments to estimate the benefits of possible regulatory actions.

Q. What research steps would you like to see next?

A. A number of subjects for research that resonated with me were raised at the meeting, and I will just highlight and elaborate on one. I would like to see research focus on how to manage the integration and interpretation of the large volume of emerging findings from the various relevant fields—medicine, informatics, basic biology and applied epidemiology and toxicology, and demographics. Data relevant to biologic variability in response to environmental stressors are being generated at different levels of biologic organization and at a deep and specialized level in different scientific disciplines, and the volume of data is enormous. The individual is a complex biologic system, and at the population level the complexity is magnified. Research on institutional and other structures that would facilitate progress to answer key questions related to public-health interventions in the face of such complexity is at the top of my list.

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(PBPK) modeling, or pharmacodynamic modeling. The last step is to derive a human equivalent dose for a sensitive population by using approaches similar to those noted above for an animal-to-human derivation. Chiu suggested three ways in which human variability data could be used to improve risk estimates, given the current risk-assessment paradigm: to develop default empirical human variability factors (in the absence of chemical-specific data), to derive chemical-specific or end point–specific variability factors, and to develop biologically based models that incorporate human variability.

Dourson, Clewell, and other meeting participants advocated for the development and use of chemical-specific adjustment factors (CSAFs) in lieu of default uncertainty factors. The World Health Organization's International Programme on Chemical Safety first developed the concept of CSAFs to have an agreed-on quantitative process for replacing the usual uncertainty factor of 10 with a factor that is more chemical-specific, Clewell said. The CSAF for toxicokinetic variability is based on a comparison of a directly measured or modeled surrogate for an internal exposure with a compound. Examples are the comparison of the area under the dose–response curve for an animal with that for a human and the comparison of an average "normal" person with a more sensitive person or population.

Pharmacokinetics vary in a population because of a number of interacting factors, such as height, weight, body fat, and health status, Clewell explained. He emphasized that it is particularly important to consider population variability when studying early life. With PBPK modeling, toxicologists can incorporate the wealth of data on age-dependent changes in organ weights, blood flows, and other well-studied biologic and biochemical processes into a model whose parameter values change with age. After determination of which enzymes metabolize the chemical of interest, it is possible to use a ratio to estimate early-life values on the basis of adult levels and to model the blood concentration of the chemical at different ages for the same exposure dose.

Clewell discussed data that illustrate average blood concentrations of two compounds, tetrachlorodibenzodioxin (TCDD) and nicotine, over the course of a human lifetime. Nicotine, which is water-soluble, mimics what you generally see with water-soluble drugs: exposure early in life tends to be proportionally greater than exposure of adults because of the ontogeny of the enzymes responsible for the clearance of the chemical. The time course for TCDD, which is highly lipophilic, is much more complex because a number of factors become important at different ages, he said.

Clewell also described an approach to modeling of population variability in toxicodynamics that is based on individual-level in vitro data. The National Institutes of Health is actively pursuing the use of induced pluripotent stem cells from a large number of people to investigate variation in susceptibility to disease. The same technology can be applied to investigate human individual variability in toxicodynamics. Clewell is working with induced pluripotent stem cells to see to whether they might offer a way of looking at population variability in susceptibility to chemicals

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Risk Assessment–*Speak*[1]

Benchmark dose: a dose that produces a predetermined change in the response rate of an adverse effect in comparison to background.

Dose–response assessment: the component of risk assessment that examines the relationship between exposure to different doses of a substance and their effects.

Hazard identi f ication: the determination of whether a stressor has the potential to cause harm to humans or ecologic systems and, if so, under what circumstances.

Risk assessment: the process of characterizing the nature and magnitude of health risks to humans or ecologic receptors posed by chemical contaminants and other stressors in the environment.

Uncertainty factor: a default factor (usually 10) used to derive reference doses or concentrations (doses or concentrations of exposure that are likely to pose no appreciable risk of deleterious effects during a lifetime) from experimental data. Uncertainty factors are used to account for such characteristics as variations in susceptibility among members of a population and uncertainty in extrapolating animal data to humans.

[1] Definitions are based on the EPA risk-assessment glossary available at http://www.epa.gov/risk/glossary.htm .

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by using cells that are in some sense normal.

Rothman noted that his research on overlaying multiple risk factors for bladder cancer also calls into question whether a safety factor of 10 is adequate. He predicted that studies similar to the one he described will uncover groups whose susceptibility is more than 10 times greater "very soon." From a public-health regulatory perspective, Rothman said, the goal is to think about the whole population to make the workplace safe for everyone, not just for the least susceptible. Many meeting participants agreed that the data presented by Rothman and others made a good case for using chemical-specific adjustment factors more widely. Dourson commented that if toxicologists have amassed chemical-specific data on a given substance, "we expect them to use them" for risk assessment.

> ***Traditional risk-management processes can consume considerable resources with little clarification of uncertainties, especially when there is large individual variability.***
>
> **—Nicholas Ashford**

Chiu also thought that human variability data could inform pathway-based approaches to dose–response assessment, as characterized by the 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy.* That report championed the concept of identifying and testing *toxicity pathways*, biologic pathways that, when sufficiently perturbed by an exposure, lead to toxicity or disease. Chiu asked whether panels of in vitro (cellular) assays could be used to assess individual variability in a high-throughput manner that would be consistent with the vision of the National Research Council report.

Nicholas Ashford, of the Massachusetts Institute of Technology, cautioned that an overcomprehensive and protracted risk-assessment process may unjustifiably postpone the implementation of desirable risk-reduction measures. He contended that a more synchronized risk-management process is needed. Rather than the sequential process currently used, he suggests a dual parallel approach for clarifying risk information and generating information about safer technologic alternatives. He also argued that if the technologic alternatives are substantially different, rather than marginally different, comparative, rather than full, risk assessments can be used. He suggested that chemical structure–activity relationships could be especially useful in such cases.

At the end of conducting a risk assessment of a compound, regulators try to identify critical uncertainties, said William Slikker, of FDA's National Center for Toxicological Research. With each review cycle, FDA risk assessors look at the literature that has been published since the last time a risk assessment or a review of a particular pollutant was conducted. Assessors are sometimes frustrated by finding that the followup needs that they identified in previous reviews "got lost in the documentation," he said. He pondered how to bridge the gap between the academics who often conduct the research and the risk assessors who conduct reviews—how to inspire both to investigate issues that could resolve key uncertainties and recognize that additional details on populations could be valuable in refining risk estimates. Slikker believes that the research and development arm of EPA does a good job of trying to bridge those communities, but the dots are not always connected.

Implications for Personal Health Decisions

Advances in tools and approaches to measure human variability have implications beyond regulatory risk assessment. Peter Shaw, of Merck, discussed how improved information about human variability is helping the pharmaceutical world to develop more targeted therapies—in other words, personalized medicine. The optimal situation, he said, is "when you understand the biology at the start of drug development, and you have a target that either is expressed in a fraction of the population or is active in a population." In the optimal situation, both a drug and tests to identify populations that can benefit most from the drug can be developed at the same time. Shaw named several cancer treatments for which the optimal situation occurred—trastuzumab for breast cancer, crizotinib for non–small-cell lung cancer, and vemurafenib for late-stage skin cancer. But typically there is insufficient biologic evidence "to associate a molecular marker with a drug response" at the beginning of drug development, he said. Often,

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genetic or metabolic data about who may be best suited to receive a specific treatment is discovered after a drug has been approved by FDA. At that point, Shaw said, it is difficult to change clinical practice even when it is clear that some patients will benefit more than others from specific treatments. As a result, the pharmaceutical industry "is under pressure to produce medicines with improved benefit:risk profiles." New advances in genomic and health-information technologies are facilitating the pharmaceutical industry's ability to develop more personalized medicines.

Barbara Biesecker, of Johns Hopkins University and the National Human Genome Research Institute, discussed human variability in the context of genetic counseling. She said that genetic counselors help people to make personal health decisions—whether to continue pregnancies, whether to face a biologic risk and have more children, and whether to learn about their risk of the diseases for which there are genetic tests. But genetic risk assessment is based largely on rudimentary tools, such as family history or a specific phenotype, she said. The tools are limited in that they fail to include all risk factors, because many are still unknown. Biesecker emphasized that even when someone has a recognized pathogenic mutation in a known gene associated with disease, there remains variability in whether the person will develop the disease. She expressed excitement about how researchers are combining environmental and genetic factors to predict disease risks more accurately. It is unclear what the future paradigm for developing guidelines to interpret and provide genetic risk assessment will be, Biesecker said. We need to determine whether the information mediators will be health-care providers, health-care systems, regulators, the public, or sets of people who have been identified as at increased risk. Then we can begin to figure out how to communicate what they need to know because each group will require different approaches, she said.

Moving Science Forward

Meeting participants discussed a variety of avenues to consider as the science on human variability moves forward. "We are clearly at a point, in terms of what kind of targeted research can be conducted, to advance this science," Farland said. Data integration and interdisciplinary problem-solving will both be important, he emphasized. Richard Woychik, of the National Institute of Environmental Health Sciences, called for a true systems-biology approach. Currently, research silos, including the genomics people who are sequencing genomes to find things like SNPs and experts in proteomics and transcriptomics, believe that they are conducting systems biology. However, systems biology encompasses everything that all these experts are doing, and we need better integration among different disciplines, he said.

Deborah Winn, of the National Cancer Institute, remarked that a large human population study with vast amounts of data on exposures, individual susceptibility factors, and multiple health outcomes is needed. In epidemiology, we often worry about generalizability, she said, but there may be times where we would benefit from focusing on groups, such as breast-cancer survivors or women who are at high risk for breast cancer. Nsedu Witherspoon, of the Children's Environmental Health Network, added that characterizing the range and distribution of biologic variability in children will help to protect both children and the general population.

Jim Kaput, of the Nestlé Institute of Health Sciences, asked how population studies can be designed to look at gene–environment interactions. If you look at genetic diversity maps, it is clear that most of our case–control studies probably lack sufficient power to detect differences because of genetic heterogeneity of populations, Kaput argued. He suggested that evaluating metabolic variability may be a better method for separating participants on the basis of responses to an intervention. He also emphasized that nutrition is an important aspect of the environment that bears on individual variability but often is not measured. Food compositions vary depending on where you grow the food, how you process it, and how you cook it; and there are bioactive substances in food that alter the expression of genes that are involved in the metabolism of toxins, drugs, and nutrients, explained Kaput. So you can have chemicals in food—such as fatty acids, sterols, and sterol esters—that bind transcription factors and alter the expression of genes that we know about. But when we measure toxic effects and drug effects, we rarely, if ever, measure nutritional environment, he said.

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Toxicant-Induced Loss of Tolerance

–by Kellyn Betts, edited by National Research Council staff

Individual variability in response to chemical exposures may play a role in explaining why some people report being very intolerant of or susceptible to the presence of chemicals in their environment. Claudia Miller, a professor of environmental and occupational medicine at the University of Texas Health Sciences Center in San Antonio, told meeting attendees that what she calls "toxicant-induced loss of tolerance" affects millions of people around the globe.

Miller's research shows that the intolerance begins with an event, such as exposure to cleaning agents or pesticides. Some people who are exposed to such agents develop what Miller calls "loss of specific tolerance" and begin to respond more intensely to exposures to extremely low concentrations of substances in air, food, and drugs that did not bother them previously and do not generally affect most people. Miller has documented the syndrome in military personnel, industrial workers, people living in communities where they were exposed to chemicals, people exposed to chemicals in their homes, and occupants of so-called sick buildings.

Most people spend about 90% of their days indoors, and indoor air can include some unusual chemicals, Miller said. For example, complex mixtures can form indoors as a result of such phenomena as interactions of different volatile organic chemicals with each other and adherence of ozone to particles.

In the United States, near 15% of the population reports chemical intolerances, and 5% are afflicted by severe sensitivity that dramatically affects their lives. Cases have been documented in an array of demographic groups in more than a dozen countries.

Miller has developed a tool that she calls the Quick Environmental Exposure and Sensitivity Inventory (QEESI), which is used clinically in many countries to identify patients. The tool can be self-administered. A second tool for working with patients who have toxicant-induced loss of tolerance is the Environmental Medical Unit, Miller said. These units do not yet exist despite multiple recommendations by Congress, the National Research Council, and professional organizations, but Miller described how they would be constructed from materials that do not out-gas. Appropriate materials include granite floors and walls and porcelain ceilings, and the units would have an optimal ventilation rate. "Our achievements in genomics, proteomics, and so on over the last 20 years have only heightened the enormous potential of such a facility to help us understand the biologic effects of chemical exposures and the subtleties of individual exposure," she concluded.

> ***Just as adverse reactions to drugs have increased with the increased use of pharmaceuticals, we are seeing the phenomenon of chemical intolerance increase as xenobiotics increase in our environment.***
>
> **—Claudia Miller**

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Farland noted that meeting presentations and discussions had made it clear that scientists need to do a better job of characterizing variability. "Susceptibility needs to be discussed in the context of variability," he said—both the quantitative and qualitative differences in susceptibility. New technologies may pave the way forward, but we must be careful "not to trade knowns for unknowns," he cautioned. Farland also urged participants to move beyond arguments about "whether a safety factor of 10 is great enough." Future environmental-health research needs to address sources of uncertainty. The science and discussion should focus on the "differences that we have not yet recognized because of our lack of understanding of variability but that would cause us to take different approaches to decision-making or communicating with the public," Farland said in closing.

Presentations and Discussions from the **Biologic Variability meeting** are available at http://nas-sites.org/emergingscience/meetings/individual-variability/

National Academies Reports on Risk Assessments

- Risk Assessment in the Federal Government: Managing the Progress (the "red book," 1983)
- Science and Judgment in Risk Assessment (1994)
- Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment (2007)
- Toxicity Testing in the 21st Century: A Vision and Strategy (2007)
- Phthalates and Cumulative Risk Assessment (2008)
- Science and Decisions: Advancing Risk Assessment (the "silver book," 2009)

The National Research Council has published many reports on risk assessment, beginning with the 1983 "red book," *Risk Assessment in the Federal Government: Managing the Progress,* through the more recent "silver book," *Science and Decisions: Advancing Risk Assessment,* in 2009. Meeting participants referred to these books as laying the framework for the use of molecular information to inform science-based toxicity decisions. To download free PDF copies of these books or to purchase them in hard copy, please visit http://www.nap.edu/.

ESEH on YouTube

www.youtube.com/EmergingScience/

Beginning in 2013, all videos from emerging science meetings will be accessible through a dedicated YouTube channel. Please check out the presentations and discussions from our most recent meeting on *Integrating Environmental Health Data to Advance Discovery.*

Gulf of Mexico Program on Environmental Protection and Human Health

The U.S. Department of Justice recently announced two legal settlements arising from the 2010 Deepwater Horizon disaster. BP Exploration and Production, Inc., and Transocean Deepwater Inc., the operator of the oil drilling platform, have agreed to pay the federal government $4 billion and $1.4 billion, respectively, in civil and criminal fines. Under the settlements the National Academy of Sciences (NAS) will receive a total of $500 million to establish a 30-year Gulf of Mexico (GoM) program. The GoM program will draw upon the nation's science, engineering, medical, and public health expertise to conduct studies, projects, and other activities that will contribute to the protection of human health and environmental resources in the Gulf of Mexico and on the United States' outer continental shelf.

The Coast Guard attempted to burn off oil leaking from the sunken Deepwater Horizon rig, April 28, 2010.

Chris Elfring, the former Director of the Board on Atmospheric Sciences and Climate and the Polar Research Board within the National Research Council of the National Academies, is the director of the new GoM program. Chris is one of the NAS's most seasoned board directors and will bring to her new role sound judgment and enthusiasm for this new endeavor. Under Chris's directorship, the GoM program will be conducted solely at the direction of the NAS, based on scientific merit and integrity with emphasis on freedom of inquiry and independent, nonpartisan advice and recommendations. The settlement calls for the GoM program to engage in three areas of work: research and development, education and training, and environmental monitoring. Among its activities, the Gulf program will fund projects in the public interest. Neither BP nor Transocean will be involved in any decisions related to the program.

MEETING INFORMATION

Meeting Presentations

Would you like more details about individual variability or other Emerging Science for Environmental Health meeting topics? Descriptions, agendas, presentations, and newsletters for our meeting topics are available through our Web site. We invite you to subscribe to our Listserv for the latest information about upcoming meetings, available newsletters, and other Emerging Science for Environmental Health activities. For more information, visit:

http://nas-sites.org/emergingscience/

Next Meetings

June 6–7, 2013 Biological Platforms

September 19–20, 2013 Topic to be determined

Do you have an idea for a meeting topic? We would love to hear it. Please send us an email with your suggestion at eseh@nas.edu.

Previous Meetings

Integrating Environmental Health Data to Advance Discovery —January 10–11, 2013

Exploring Human Genomic Plasticity and Environmental Stressors: Emerging Evidence on Telomeres, Copy Number Variation, and Transposons — October 4–5, 2012

Biological Factors that Underlie Individual Susceptibility to Environmental Stressors — April 18–19, 2012

Emerging Technologies for Measuring Individual Exposomes — December 8–9, 2011

Applying 21st Century Toxicology to Green Chemical and Material Design—September 20–21, 2011

Mixtures and Cumulative Risk Assessment: New Approaches Using the Latest Science and Thinking about Pathways —July 27–28, 2011

Interplay of the Microbiome, Environmental Stressors, and Human Health —April 27–28, 2011

The Use of In Utero and Post-natal Indicators to Predict Health Outcomes Later in Life — October 14–15, 2010

Stem Cell Models for Environmental Health —June 3–4, 2010

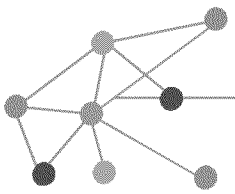
The Exposome: A Powerful Approach for Evaluating Environmental Exposures and Their In f l uences on Human DiseaseFebruary 25–26, 2010

Computational Toxicology: From Data to Analyses to Applications —September 21–22, 2009

Use of Emerging Science and Technologies to Explore Epigenetic Mechanisms Underlying the Developmental Basis for Disease —July 30–31, 2009

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies forms the Standing Committee on Use of Emerging Science for Environmental Health Decisions to facilitate communication among government agencies, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.



EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS NEWSLETTER

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Diversity Outbred

A New Generation of Mouse Model

The use of genetically identical mice in toxicology studies can make it tricky to extrapolate findings to people. A new mouse model known as the Diversity Outbred better reflects the genetic diversity of the human population, offering intriguing possibilities for safety assessment. © Roy Scott

Most of the mice used for testing the toxic effects of chemicals and drugs are genetically inbred with a long history in the laboratory.[1] But toxicologists are increasingly turning to newer mouse models that more accurately mimic the genetic diversity of the human population. Investigators with the National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences have now reported that one such model—the Diversity Outbred (DO) mouse model—varies widely in its susceptibly to benzene, a known cause of human leukemia.[2] The results demonstrate the model's improved capacity for identifying subtle chemical effects and lend further credibility to the use of DO mice in toxicology research and safety assessment, according to lead author John E. French, a toxicologist specializing in toxicogenetics formerly with NTP and now an adjunct professor in the Center for Pharmacogenomics and Individualized Therapy at the University of North Carolina at Chapel Hill.

Proof of Concept

Because toxicity depends in part on how chemicals and genes interact, genetically inbred mice—generated by breeding siblings—tend to respond similarly to the agents tested in a given study. That has certain advantages; for instance, it limits the number of animals needed to detect statistically significant differences in chemical effects. But among other disadvantages, it's possible that inbred mice might exhibit strain-specific responses with little relevance to the genetically diverse human population, says Kristine Witt, a toxicologist with the NTP.[3]

It's not unusual for toxicologists to work with outbred mouse strains derived from unrelated pairings. These strains have more varied reactions to chemicals and drugs, but they also vary unpredictably with respect to their own "outbredness." By contrast, the DO model is maintained under strict randomized breeding conditions designed to ensure that only unrelated mice mate.[4,5] Thus, every DO mouse is genetically unique. Moreover, the eight "founders"—the original parental strains of mice from which all subsequent DO generations derive—were fully sequenced,[6,7] "and so we can reconstruct the genome of any single DO mouse with a high degree of precision," says Gary Churchill, a professor at Jackson Laboratories in Bar Harbor, Maine. That ability, Churchill says, facilitates genomewide association studies that aim to pinpoint the genes or alleles that govern a particular trait.

For the new proof-of-concept study,[2] NTP investigators and their collaborators exposed two independent cohorts of 300 male DO mice each to benzene. This chemical was chosen because its metabolism *in vivo* is well characterized and known to be similar in mice and humans. "The possibility of finding distinct gene associations in the response to benzene exposure, based on the diversity of the metabolic pathways involved, seemed high," says Witt, a coauthor.

Groups of 75 mice each were exposed to benzene in air at 0, 1, 10, or 100 ppmv for 28 days. Then the investigators looked at peripheral blood and bone marrow samples for evidence of micronuclei (MN). MN arise from chromosomal fragments or whole chromosomes that fail to incorporate into daughter nuclei during cell division, and their numbers are known to increase dose-dependently with benzene exposure.

MN counts in peripheral blood were significantly different in mice with the highest exposure compared with unexposed animals, but were similar to unexposed mice for those animals with lower exposures. MN counts in bone marrow, however, differed from nonexposed controls at every dose level.[2] "We can't sample the bone marrow in exposed humans, but these results suggest that changes in blood may not reflect bone marrow toxicity among the most sensitive individuals," French says.

Like DO mice, humans differ in their susceptibility to benzene, with some showing evidence of blood toxicity at exposure levels below the federal occupational standard.[8,9,10,11,12] Importantly, though, the benchmark concentration was an order of magnitude lower than the concentration estimated in earlier studies with inbred B6C3F1 hybrid mice, which have been used routinely by the NTP since the 1970s and are still in widespread use today.[2] The benchmark concentration is the concentration associated with a small but measurable biological response—in this case, at most a 10% increase in micronucleation compared with nonexposed animals.

When the investigators repeated the same experiment four months later, they got the same results. As before, individual DO mice varied in their response to benzene, but the cohorts' overall variation was very similar to that seen in the first study.[2]

"There was no statistical difference between the data sets," Witt says. "All the exposed mice were each genetically different from the others, with different coat colors and temperaments—just like humans. But even so, our results were reproducible. This observation was crucial for convincing the toxicology community that DO mice can be a useful tool." If the two data sets had been wildly different, she says, then the DO model would not be seen as reliable for chemical testing.

By performing linkage analyses on the mouse genomes, the investigators were able to home in on genes that confer resistance to benzene toxicity—most likely a group of two sulfotransferases located on chromosome 10 that modify and eliminate benzene metabolites.[2] Witt says the sulfotransferases could modify benzene metabolites in ways that limit their ability to reach or harm bone marrow, the source of the blood stem cells that can give rise to benzene-induced leukemia. Humans have analogous sulfotransferases that are known to have similar activity. She says, "This illustrates how genetic results from toxicity studies in DO mice can guide us toward related genes in humans for further study and can help elucidate underlying mechanisms of action leading to toxicity and disease."

Michael DeVito, acting chief of the NTP Laboratory, says DO mice could help toxicologists ensure that they don't miss a potentially significant human end point. He says the NTP is now working to better characterize the animals with respect to baseline differences in serum chemistry, organ weights, reproductive capacity, and other measures, with the anticipation that the model may eventually be incorporated into NTP testing protocols. "The more of these studies we do, the better will be our understanding of the normal population variation," French says.

The Founders

The DO mice were created during the last decade from a predecessor model called the Collaborative Cross (CC).[13,14,15] Efforts to create the CC date back to 2002.[16] David Threadgill, a geneticist and professor at Texas A&M University, says scientists at the time had become increasingly aware that genetic background can dictate phenotype in toxicology. Worried that they might be missing important human end points by relying on established inbred strains in research, Threadgill and other scientists created the Complex Traits Consortium (CTC) with a mission, he says, "to reinvent the mouse model so that it would contain genetic variability on the scale of what exists in humans."

To accomplish that mission, the CTC crossbred eight founder strains from the three major laboratory and wild subspecies of *Mus musculus*, otherwise known as the house mouse. Analyses confirmed that the eight strains captured 90% of the genetic variation known to exist in *M. musculus*, and that the variation was randomly distributed across the genome.[13,17] The eight strains were crossbred using a "funnel" design that sequentially narrowed generational matings. Eventually, siblings were mated to generate inbred strains, "each with a random sampling of the genetic variation that was initially present in the founders," Threadgill explains.

According to Churchill, inbred CC strains are defined on the basis of two criteria: Their genomes must contain DNA from at least six of the eight founders, and they must display 98% homozygosity, meaning the copies of each gene inherited from the mother are identical to the copies inherited from the father.

To maximize access to the founders' genomic diversity, scientists experiment with as many different CC strains as they can, DeVito says. This approach was illustrated in a landmark 2014 paper by researchers who worked with 47 CC strains and found that they exhibited varying reactions to the Ebola virus, just as humans do.[18] Traditional inbred mouse models don't develop the human-like symptoms of Ebola hemorrhagic fever, which include delayed blood coagulation, intravascular blood clots, and potentially death from shock. But according to this widely reported paper, some CC strains do exhibit these symptoms, with lethality in the animals dependent on genetic background—susceptible animals showed 10- to 100-fold increases in the expression of genes that induce inflammation, cell death, and vascular leakage. By contrast, genes that limit vascular leakage, possibly by facilitating repair of blood vessels, were upregulated in resistant mice. Genetic factors may therefore play a significant role in determining human survival of infection with the Ebola virus, the authors speculated.[18]

According to Churchill, the CTC's initial goal was to breed up to 1,000 CC strains. Yet that proved unfeasible because so many of the strains died out over time. "We ran into fertility problems," Churchill explains. "After about five generations, ninety percent of the strains would stop producing pups." That was, to some extent, a predictable setback, Churchill adds, given that inbred animals often suffer from health problems and poor reproductive capacity.

Still, some CC strains bred vigorously, and the panel now comprises roughly 200 recombinant inbred strains, of which 90 currently are publicly available; as the remaining CC strains are inbred, they will be released to the public, Threadgill says. Those strains will ideally contain the genetic variation researchers need to map the genes they're looking for in a given study—for instance, genetic traits that might predict outcomes among Ebola patients. "But luck also plays into the game," Churchill says. "If you go through all the available CC strains and you still come up empty-handed, then you've hit a wall."

Developing the DO Model

That limitation is what galvanized scientists to develop the DO mouse model in 2009.[4,19] To generate DO mice, scientists randomly breed across the different CC strains. Random mating minimizes the potential for genetic drift, or the loss of genetic variety in

the population, Threadgill explains. Thus, genetic diversity is broken out into finer and finer scales, and according to Threadgill, this allows for far more resolution in genetic analysis than is achievable in CC strains with a fixed genetic structure.

Upon finding the genes that govern a particular trait in DO mice, researchers can then check to see if those genes are also present in a given CC line. This is important because it's impossible to reproduce genetically identical DO cohorts. Since all the animals in a given cohort are genetically unique, researchers have no way of knowing if genes of interest found in one group of DO mice will also be present in another group. But if those same genes can be subsequently identified in a CC strain, then that strain can be continually replenished for ongoing research. In that sense, Churchill says, the DO and CC models complement each other—researchers can hunt for genes in DO animals, and then go on to study the genes they find in a renewable pool of CC mice.

Still, DO mice pose a fundamental challenge to research and testing: Because it's impossible to know which animals have the genes and allelic variants of interest, researchers by necessity have to search for them in as many animals as possible. According to Threadgill, the specific number depends on the complexity of the genetic pathways involved. "If you've got a simple pathway with just three to four genes controlling a given trait, you can get by with fewer animals," he says. "That's not true for highly variable traits controlled by lots of different genes, however."

DeVito acknowledges that sample size and statistical power requirements with DO mice are open questions at the NTP. To gain a better understanding of their physiology, DeVito and his colleagues recently launched a pilot study. They put 75 DO mice on a high-fat diet, and then compared changes in serum chemistry, histology, organ and body weight, and other end points with those of control DO animals fed normal diets. Unpublished results showed that individual animals from either group differed little with respect to these end points, except for sperm counts, which varied tremendously in both the control and high-fat groups for unknown reasons.

"It's not like we had a few extreme outliers," DeVito says. "Instead, the sperm counts rose gradually among the animals, with a seventyfold difference between the lowest and the highest values."

For context, DeVito points out that B6C3F1 mice normally have no more than a twofold difference in sperm counts. The fact that the counts vary so widely in DO mice presents research difficulties, especially for studies of male reproductive toxicants. Instead of using 10–20 animals per treatment group, which is what NTP guidelines recommend in studies with inbred strains, scientists would probably need to use hundreds of DO mice to pick up subtle reproductive effects that could be distinguished from results in untreated controls, according to DeVito.

"Traditional study designs will not have the same statistical power in the DO as they do in more typical inbred strains," DeVito says. "We need to better understand the variability in the untreated DO mouse for any end point so that we can appropriately design a study for this model."

All that said, DO and CC mice both offer promising opportunities for chemical risk assessment, says Weihsueh Chiu, a professor at the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University. According to Chiu, DO and CC mice offer three fundamental benefits: 1) they improve hazard identification by allowing scientists to pick up toxic effects that might not be evident in a resistant inbred strain; 2) they improve dose–response assessment by modeling human genetic diversity; and 3) they improve mechanistic understanding through techniques such as genomewide association studies to identify potential pathways governing toxic resistance or susceptibility to toxicity.

But Chiu acknowledges that the benefits of genetic variability come with a trade-off. DO and CC mice are more expensive than other laboratory mice, Chiu notes, and costs must be balanced with statistical power requirements, echoing the study design issues raised by DeVito.

"The essential question is this: In what cases do the benefits in terms of hazard identification, dose response, or mechanistic understanding justify the additional costs of using DO or CC mice?" Chiu asks. "Right now, we have proof of concept that they can be useful. We're in a development and refinement stage, and I'm confident that in the process, we can figure out how best to use them to support our ultimate goal of protecting public health."

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Expanding the Scope of Risk Assessment: Methods of Studying Differential Vulnerability and Susceptibility

Several methodological issues have been identified in analysis of epidemiological data to better assess the distributional effects of exposures and hypotheses about effect modification.

We discuss the hierarchical mixed model and some more complex methods. Methods of capturing inequality are a second dimension of risk assessment, and simulation studies are important because plausible choices for air pollution effects and effect modifiers could result in extremely high risks in a small subset of the population.

Future epidemiological studies should explore contextual and individual-level factors that might modify these relationships. The Environmental Protection Agency should make this a standard part of their risk assessments whenever the necessary information is available. (Am J Public Health. 2011;101:S102–S109. doi:10.2105/AJPH.2011.300367)

Joel Schwartz, PhD, David Bellinger, PhD, and Thomas Glass, PhD

IN OUR FIRST ARTICLE IN THIS supplement,[1] we identify several critical concepts that need to be incorporated into risk assessment to adequately address differential vulnerability and susceptibility to environmental hazards. In our second article,[2] we illustrate these concepts, drawing examples primarily from the literature on lead exposure and air pollution. Here, we discuss methodological issues arising from our recommendations in those articles. Several issues are not addressed here, such as problems of measurement; a rich literature on measurement issues in lead research is available.[3–7] We focus on issues related to the study of differential vulnerability and susceptibility. This research faces 3 core methodological challenges, but existing, new, and emerging methods can address them. These challenges are (1) complex interactions and synergies, (2) nested data at multiple spatial scales, and (3) methods to quantify risk inequality to identify hidden pockets of vulnerability.

COMPLEX INTERACTIONS AND SYNERGIES

Certain standard assumptions underlie the risk assessment approach: independence (discrete exposures are independent of one another), risk averaging (a single overall scalar estimate of average risk is adequate for decision-making), and risk accumulation (the potential for complex distributions that arise from a multirisk exposome). The risk assessment approach needs to expand to account for complex interactions and synergies that exist between multiple exposures and between other important biological and social variables that may place individuals or population subgroups in a higher state of vulnerability or susceptibility.

The most widely used methodological approach to the study of differential vulnerability and susceptibility is the use of regression models to test hypotheses about effect modification, by either stratification or interaction terms. Effect modification occurs when the effect of some exposure on outcome varies by or depends on the value of some other variable. Effect modification is a causal as opposed to a statistical concept, which implies that causal associations are contingent or dependent on 1 or more other variables. Many examples in the published literature show that the effects of environmental exposures vary according to both individual[8–12] and community characteristics.[13]

One implication of effect modification is that a summary effect estimate may be incomplete or misleading because if the effect of exposure varies by a third variable, no single effect estimate can accurately capture pools of heterogeneous effect. If the magnitude of an association between an exposure and an outcome varies across strata of a third factor, an estimate that summarizes the association across strata of this factor will overestimate the association in a stratum in which the association is absent and underestimate it in a stratum in which it is present.[10,14] In extreme examples, a deleterious effect can be entirely masked when the relevant effect modifiers are not taken into account. Effect modification is not the same as confounding, although both are causal concepts. Effect modification is a property of a true association and not a consequence of a distortion in an association masquerading as a causal effect.[10]

Several authors have written about the limitations of interaction terms in the study of effect modification, including Vineis and Kriebel,[15] Cox,[16] and Greenland.[17] At least 5 important limitations of this approach should be considered. First, a host of potential functional forms are possible and must be correctly specified for interaction terms to adequately capture the nature of the causal relations at play. Although investigators may be sensitive to the possibility of 2-way interactions that are either additive or multiplicative, these are only 2 among many complex forms of interaction that should be evaluated, such as the possibility of nonlinear interactions. Second, stratification explicitly or through interaction terms reduces power, which can increase the likelihood of unstable estimates. Third, interaction terms to specify complex causal interactions are limited to a narrow range of dimensionalities. To the extent 2 risk factors combine to produce an etiologic effect that is different than the additive or multiplicative effect of the 2 variables, more complex approaches

such as thin plate splines and random forests may be necessary, and without sufficient power, even these models may be inadequate. Fourth, when our data are snapshots of complex, dynamic life course processes, statistical interaction may be ineffective at capturing the dynamics of risk amplification. This is implied by the concept of a developmental window of vulnerability, which can lead to complex interactions that appear and disappear depending on when exposure and effect modifiers are measured. Finally, linear models are not well suited to differentiating variables that are effect modifiers rather than mediators on the causal pathway.[18]

Although regression approaches that use interaction terms represent a powerful and important set of tools for the evaluation of complex interactions and synergies, other tools are available. Recently, marginal structural models and inverse probability-of-treatment weighting have been used to examine effect modification.[19,20] This approach is effective in studying dynamic life course developmental processes, where the value of either exposures or effect modifiers is known to be time varying.[21] Berkey et al. demonstrated the use of multilevel random-effects models for estimating effect modification across places,[22] an approach well illustrated in an analysis of EM effect modification in 29 European cities in the APHEA2 project.[14]

A potentially powerful set of alternative methods comes from systems analysis, a flexible method of examining higher-dimensional interactions that include nonlinearities and feedback loops.[23–26] Systems dynamics models have been implemented most widely in infectious disease epidemiology and only rarely to study environmental exposures. Finally, a host of general approaches characterized by classification and regression trees can identify clusters of jointly occurring risk factors without imposing any linear modeling assumptions.[27,28] These represent another underused tool with great potential for studying highly complex patterns of differential vulnerability.

A key issue in modeling interactions between environmental exposures and measures of susceptibility, whether social, genetic, or arising from disease status, is that the variables often exist on multiple levels, with different and crosscutting influences. For example, socioeconomic position (SEP) is a variable that can be conceptualized at the level of the individual, the family, or the community or across generations. In addition to individual-level SEP, the socioeconomic aspects of social context affect people's health and potentially their response to exposure.[29–33] Hence, a wealthy person residing in a predominantly poor geographic area may be exposed to the same risk landscape (excess of fast food, dearth of fresh produce, psychosocial hazards, toxicant exposures) as poorer residents. However, wealthy individuals with substantial resources may escape the deleterious influences of area-level socioeconomic deprivation through their greater access to more distant resources. A substantial body of evidence has shown that SEP measured at various levels modifies the influence of a variety of environmental exposures.[8,10,11,13,34] Investigators have rarely examined both area- and individual-level effect modification and how they may help define pockets of differential vulnerability across spatial and life course dimensions.

SPATIAL NESTING OF DATA

A second key methodological challenge is that sources of differential vulnerability and susceptibility may arise at higher levels of organization—in the household, neighborhood, community, or other geography of exposure. The presence of environmental contaminants may similarly vary geographically, and this spatial patterning may affect exposure. For example, within-city variation in airborne particles is predominantly driven by traffic particles, but cross-city or cross-time variations may be attributable to secondary particles, which may not be equally toxic. Similarly, soil lead declines with distance from a smelter, but some soil lead is from past emissions of leaded gasoline or lead paint residues. Toxicant exposures have generally been found to vary substantially on different spatial scales.[35–38] This supports the finding that bioavailability of toxicant exposure is geographically patterned, often at fairly small geographic scales.

Statistical modeling needs to recognize different scales of variation, both spatial and temporal. Consideration of the life course dynamics of differential vulnerability requires data collected repeatedly from individuals or across generations. This can yield multilevel data on exposure, risk factors, treatments, policies, and other relevant variables. Methods that can handle the nested nature of these data (both temporally and spatially) are critical both to accurately estimate model parameters (especially standard errors) and to provide tools to address multilevel questions about differential vulnerability. Although most studies focus on characteristics of individuals that lead to increased vulnerability, recent work points to the need to examine landscapes of risk,[39] risk regulators,[40] or the exposome[41] as geographic areas beyond individual characteristics that may be more appropriate targets of policy research and intervention. This approach requires methods that can handle complex, hierarchically nested data.

Hierarchical Mixed Models

One approach to these challenges is the hierarchical mixed model.[42–44] This broad class of models can be useful for at least 3 classes of problems where multiple measurements of each outcome are available for a individual or where data are geographically clustered within 1 or more levels. Hierarchical (or multilevel) models allow us to identify variation in baseline health across participants (via estimation of random individual-level intercepts) or differences in average levels of an outcome by geographic area (via estimation of random area-level intercepts). Of equal importance is the ability to determine variability in response to exposure (via estimation of random slopes) across either individuals or areas.

Most germane to this discussion is the ability to examine which individual- or area-level factors modify baseline levels or responses. That is, if some participants (e.g., residents of socioeconomically deprived areas) have higher blood pressures than average, the repeated measurements of those persons will all tend to be higher (or lower) than predicted and hence the residuals (measured − predicted) will all tend to have 1 sign, rather than varying randomly around zero as would be predicted if measure-to-measure variability were simply a matter of

random measurement error arising from many possible factors known to clinicians. The correlation of measured values within individuals tends to bias standard error estimates. Multilevel models correct for the nesting of repeated measures of an outcome and allow for partitioning variance within and between individuals. By fitting a random intercept to each person, the models allow for the random noise that arises with repeated measures, while capturing the correlations that arise across measures from occupancy in an area with, in this example, high levels of socioeconomic deprivation. This is important because outcome values on participants who are nested within some geographical area tend to be more alike one another; hence observations are correlated within area (which could be defined by political geography, distance from an emission point source, or catchment area of a health care system). Multilevel models handle correct estimation of regression coefficients and standard error in both types of nesting separately and simultaneously.

We may also have more complex correlations over space. In most cases, data are considered clustered by discrete administrative units that may or may not correspond to the true geography of risk. When we measure actual geographic distance between individuals and exposures, we have a host of powerful tools for breaking out of the limits of administrative geographies such as census tracts or zip codes. This is especially important for examining spatial autocorrelation in risk. Suppose the j th observation in person i and person i9 depends on the spatial distance between them. The spatial patterning of risk regimes by social status, ethnicity, and so forth may induce such a structure. In this case, empirical Bayes estimation[45,46] extensions of multilevel models can be used to account for complex patterns of spatial autocorrelation and to smooth over or fill in sparse data.

Hierarchical mixed models are easily generalized to the case of binomial outcomes such as health events[47] or rates or to survival analyses for time-to-event data,[46] but it is easiest to focus on continuous outcomes to illustrate the point. That model assumes

$$\begin{aligned} (Y_{it} &= b_0 + u_i) + \text{covariates} + (b_1 + \eta_i)X_{it} + f_{it} \\ u_i &= g_0 + gZ \\ \eta &= k_0 + kQ \end{aligned}$$

where i denotes a level of aggregation, usually participant (but census tract or year are also common), and t denotes repeated measures. Where present, u_i is the difference from the overall mean in person i, and v_i is the difference from average response to pollution (X) for person i; Z and Q are variables that explain some of the susceptibility. If i represents an individual, for example, then the variables in Z and Q may be individual level, may be neighborhood level (e.g., median household income in a census block group), or may represent periods.

Similarly, X could be decomposed, where appropriate. An example is

$$X_{it} = Z_t + (X_{it} - \bar{X}_t) + (\bar{X}_t - Z_t); \quad (2)$$

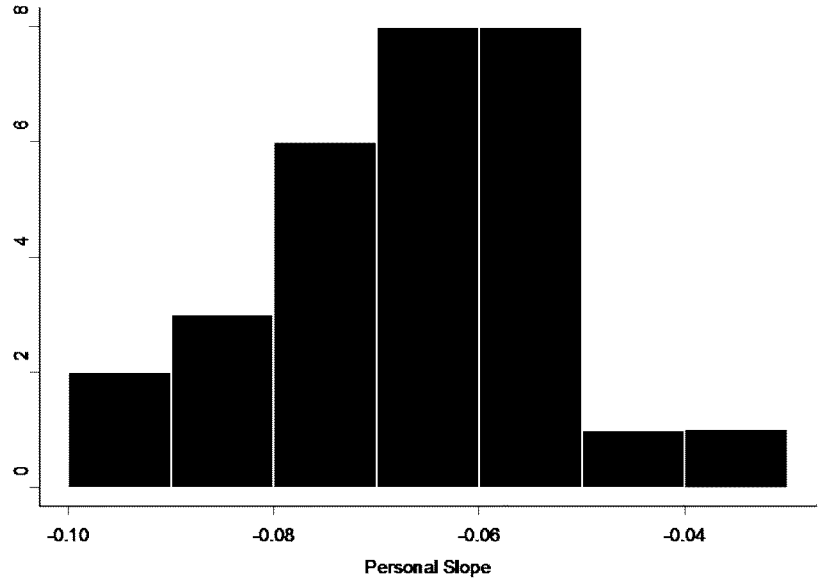
where Z_t is the air pollution reading from a central monitor, $\bar{X}_t$ is the average of the personal exposures of all the participants on day t, and X_{it} is the exposure of the ith participant on day t. In this framework, the single coefficient (b_1 in this example) is replaced with 3 coefficients—1 representing the effect of area-level pollution, 1 the effect of the difference of individual-level exposure from the mean exposure of the population on that day, and 1 the difference of population mean exposure from the monitored exposure. The second term is usually Berkson error, which, although often large, induces no bias. The last term usually includes some classical measurement error, but the first 2 can legitimately be different and tell different stories about exposure at different levels.

For example, Figure 1, taken from a repeated-measures study of air pollution and heart rate variability in an elderly panel in Boston,[48] shows the distribution of the random slopes (v_i), which is clearly skewed. Differential vulnerability is explored in Figure A of the online appendix (available a supplement to the online version of this article at http://www.ajph.org), showing that a past myocardial infarction modified the association. The modifiers in multilevel modeling can be derived from area as well as individual characteristics. For example, Zeka et al. showed that birth weight was influenced by SEP, by traffic exposure, and by interactions between the two.[49] Glass et al. used multilevel models to examine, among community-dwelling older adults, how the toxicity of lead is exacerbated by living in neighborhoods high in psychosocial hazards.[50] Figure 2 shows the use of partial residual plots to graphically display a cross-level interaction. The figure shows that the deleterious impact of lead (as measured in a tibia with ^{109}Cd-induced K-shell x-ray fluorescence) is seen only in residents of neighborhoods with high levels of psychosocial hazards. This fits well with animal models showing that stressful environments exacerbate the deleterious impact of lead on the brain.[51–53] Researchers used multilevel models to formally test the hypothesis of effect modification, which was supported in 3 of 7 domains of cognitive function examined after adjustment for individual-level confounders (age, gender, race/ethnicity, education, testing technician, and time of day).

Risk Chaining

Although standard regression methods are widely used to investigate both main and interaction effects, they rely on standard assumptions. One is that each separate predictor variable is distinct in the sense of being able to arise (or be experimentally set) without regard to the other variables entered. As described in the classic article by Gordon,[54] this property of distinctiveness derives from the larger theory guiding model building and is not simply a property of the data or study design. Risk chaining refers to the connectedness of multiple risk factors in time and space as a function of the arrangements of these variables in the world. For example, if a factory releases multiple pollutants into the air, water, and land, measurements of each individual pollutant are not distinct from one another (because they have a common source). If the correlation among those exposures is high enough, it will not be possible to treat them all as independent variables.

Similarly, areas that are socioeconomically deprived share common risk characteristics that are chained together by their common higher-level causes (racial segregation, labor market marginalization, globalization of production). This is why



Source. Schwartz et al.[48]

FIGURE 1—Relationship between black carbon and high frequency heart rate variability in a study of elderly subjects in Boston.

area-based measures of poverty, lack of education, large minority populations, and other area characteristics are highly correlated, forming a linked risk regime. In such cases, new metrics that combine multiple exposures (e.g., exposures that operate through a common biological pathway) can be generated. Alternatively, various clustering approaches can be used to identify distinct groupings of exposures, treating them as either latent or manifest constructs.[55]

Beyond regression approaches, standard linear model constraints can be relaxed and the data explored for both clustering and interactions with fewer assumptions through decision tree and machine learning approaches,[27] including kernal machines.[56] Finally, new methods drawn from engineering and computer science in systems dynamics offer ways of analyzing complex chains (or disease production algorithms) that cannot be seen because of the assumptions imposed by standard regression models.[24,57,58]

RISK INEQUITY

Risk assessment must become better at understanding sources of differential vulnerability that lead to a spatially patterned distribution of risk.[1] Studies of lead and air pollution demonstrate that social, medical, and genetic factors can modify risk.[2] Well-established methods quantify the inequality of distribution of outcomes.

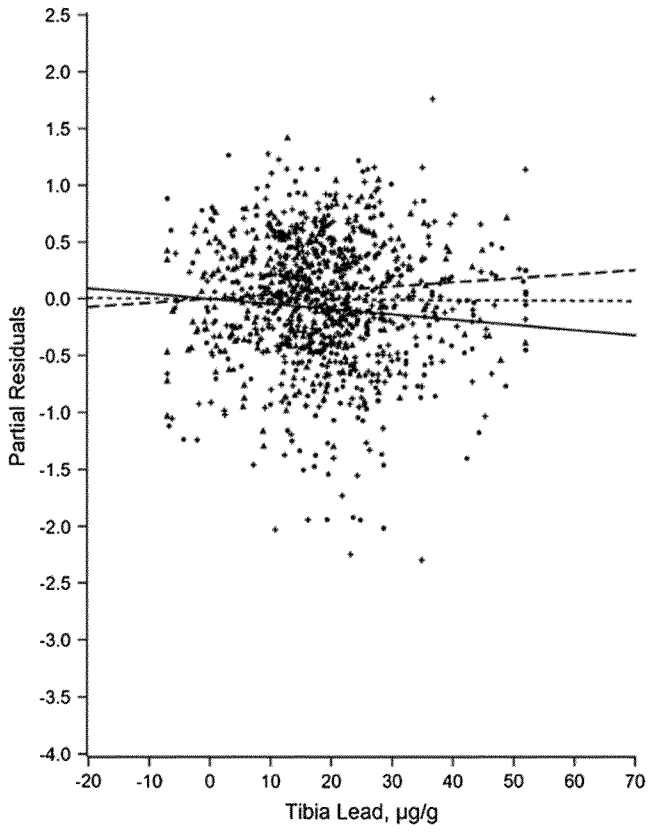
Conceptual Issues

Levy et al. quanti ed the risk reduction and equity considerations of alternative methods for reducing mortality risk associated with coal-burning power plants.[59,60] They showed alternative control strategies on 2 dimensions: e ciency (essentially risk divided by cost) and equity. They quanti ed equity with the Atkinson index, a measure of inequality in the distribution of risk. This presupposes no judgment about what an acceptable inequality is; it merely quanti es the level. By plotting multiple alternative policies on the 2-dimensional scale of e ciency and equity, this approach provides decision-makers with the necessary information to base their actions on their judgments of appropriatesocietal tradeoffs. Moreover, by making the trade-offs explicit rather than implicit, this approach encourages public discussion during rulemaking so that decisions re ect societal values.

In another approach, Su et al. adapted the concentration index from social science as a measure of inequality.[37] They used small geographic-scale units to quantify the inequality in the distribution of risk from 3 pollutants, aggregated on either a multiplicative or additive scale, and applied it to a real-world scenario in Los Angeles. Although their metric was not risk per se, but rather the ratio of risk to, for example, an ambient standard, the approach could be adopted to an absolute-risk scale, and it clearly demonstrates that distributional issues can be examined in the context of assessing cumulative exposure in the sense of multiple exposures. Other dimensions may be necessary as well. A quantification of the inequity in the distribution of risks among individuals may be insuf cient if the risks are also inequitably distributed among groups those individuals belong to. These groupings could be geographic, racial/ethnic, persons with special diets, and so on.

Examples

We constructed a hypothetical—but reasonable—scenario from the literature. The underlying risk of having a heart attack varies by income; we took strati ed risk estimates from Banks et al.[61] From the same source, we obtained estimates of how diabetes prevalence varies by income. Finally, from a recent article from Denmark,[62] we took the relative risk for heart attack among persons with diabetes to be 2.4. We then simulated the distribution of the probability of a heart attack in a hypothetical population of 1 million. We further assumed that diabetes doubles the PM (particulate matter < 2.5 Im aerodynamic diameter)—associatedrisk of heart attack (plausible because of the interactions between diabetes and at least short-term effects of particles); that 20% of the population have genetic factors, independent of diabetes, that also double the particle-associated risk; and that the risk for a 10-microgram per cubic meter increase in annual average $PM_{2.5}$ is 1.2, enabling us to examine the distribution of incremental risk.



Source. Glass et al.[50]

FIGURE 2—Partial residuals of cognitive function versus lead, with differing patterns by neighborhood level of psychosocial hazards.

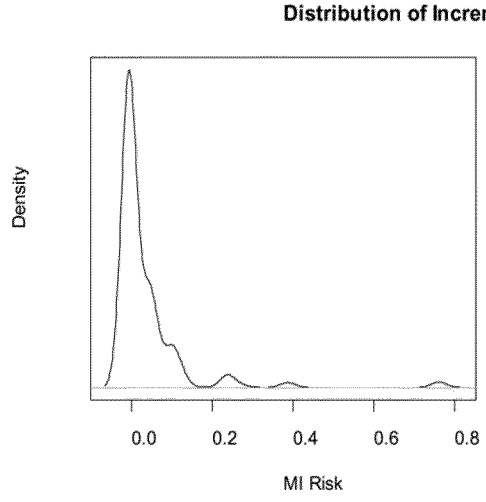
Figure B in the online appendix shows the baseline risk of heart attack in the population in the simulated scenario. Figure 3 shows the distribution of incremental risk. Although the average incremental risk is only a few per hundred (still vast compared with the risk that the Environmental Protection Agency tolerates for cancer), for a small portion of the population the incremental risk is about 0.7. Is it acceptable to impose a 70% risk of heart attack on a subset of the population? Furthermore, a single summary metric of heart attack risk overall that ignores these interlocking facets of differential vulnerability would vastly underestimate the true risk in pockets of more vulnerable subsets. These simulation results only posit additive risk accumulation. Under conditions of multiplicative or other nonlinear interactions, the results could be more extreme.

Geographic concentration of risk is also a key concern. The next figures, derived from real, not simulated, data, illustrate how this can affect equity concerns. Reid et al. examined the geographic distribution of factors shown to modify the effects of high temperatures on mortality, to produce a map of temperature vulnerability on a census tract scale.[63] Figure C of the online appendix demonstrates that geographic vulnerability varies substantially within a small area. Such neighborhood-scale variations in vulnerability cause particular equity concerns. A similar pattern is illustrated in Worcester County, Massachusetts, where Tonne et al. found a factor-of-3 range of variation in heart attack risk by census tract, again with clustering of the tracts at highest risk.[64] Figure 4, derived from their data, shows the incidence rate of heart attack in each census tract for the county as a whole and for the central area, relative to the community average rate, after adjustment for age, race, and gender.

Finally, Levy et al. examined the geographic distribution of risk of emissions from coal-burning power plants in Washington, DC. They assumed uniform risk and accounted for modification by diabetes.[65] The annual reduction in cardiovascular hospital admissions is shown in Figure D as a rate per million, assuming uniform risk in the population, then stratifying by diabetes and taking into account the differential numbers of patients with diabetes in different census tracts in Washington. Figure E is the ratio of the 2 risks. This indicates that accounting for the differential spatial patterning of diabetes and the differential vulnerability reveals substantial inequity by geography in particle-associated risk.

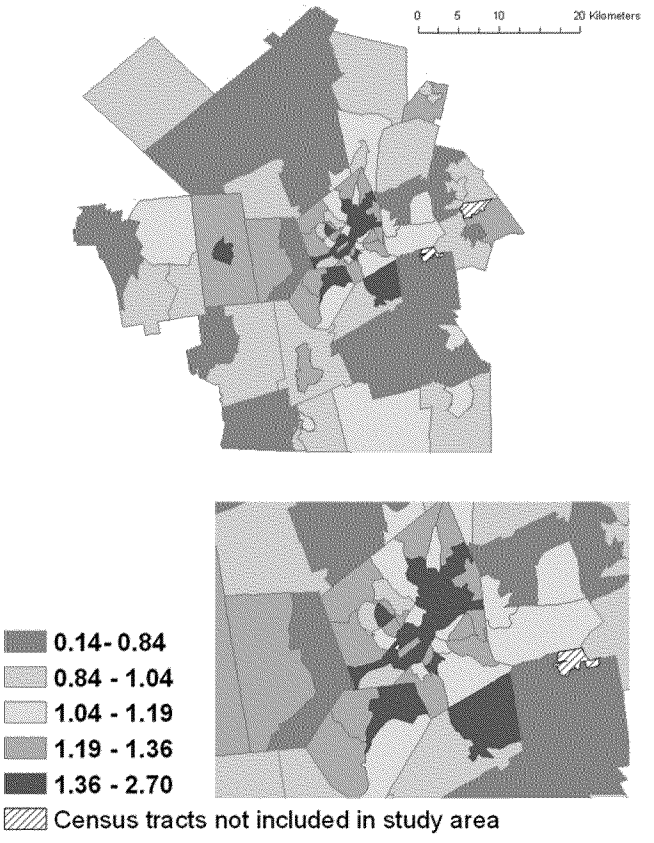
CONCLUSIONS AND RECOMMENDATIONS

If continued progress is to be made in explicating these complex phenomena, future studies of toxicant exposure—risk relationships must invest the resources necessary to measure individual and contextual factors that might modify these relationships, as well as adopting methods that allow them to estimate those impacts.



Note. There is a high incremental risk in a small fraction of the population.
Source. Based on data for lifetime risk of myocardial infarction and income from Banks et al.[61] and for lifetime risk of diabetes from Schramm et al.[62]

FIGURE 3—Simulated incremental risk of having a heart attack in the US population from exposure to $PM_{2.5}$ assuming a basic relative risk of 1.2, a 2-fold modification of risk by diabetes, and a 2-fold modification by genetic factors unrelated to diabetes.



Source. Adapted from data presented by Tonne et al.[64]

FIGURE 4—Distribution of risk of having a heart attack by census tract in Worcester MA.

Risk assessments need to move from an RfD approach to estimating attributable risk and the distribution of that risk, to allow assessment of inequity and to allow risk mangers to have quantitative measures of both overall risk and distributional aspects to inform decisions.

Environmental rulemaking is often supposed to provide protection to the population subgroup most vulnerable to a toxicant (and thus, by extension, be protective for all others). In reality, it is seldom known which subgroups are the most vulnerable or, when evidence exists, subgroup is de□ned very broadly, such as the fetus in the case of methylmercury or young children in the case of lead. Available evidence suggests, however, that not all fetuses are equally sensitive to methylmercury, nor are all young children equally sensitive to lead. If the perspective that we advocate were incorporated into epidemiology studies and subsequent risk assessments, the de□nition of the most vulnerable subgroup would become much more speci□c and therefore much more useful in targeting preventive strategies for reducing toxicant-associated morbidities. But □rst, more studies must be conducted to provide the necessary data on factors that modify vulnerability.

In most risk assessments seeking to establish an acceptable level of exposure, various uncertainty factors are applied to effect levels derived from empirical studies. These are necessary to address interspecies extrapolation (if the critical effect level is based on a nonhuman model), human variability in vulnerability (which is usually interpreted as pertaining to toxicokinetic or toxicodynamic variability), absence of data on long-term sequelae, or other gaps in the available database. The speci□c value assumed for an uncertainty factor varies, but often a generic default value of 10 is used. Most models regard this variability as stochastic and not explainable by the data. Studies should begin modeling those sources of variability with data.

Our proposal is a strategy for understanding, at a more precise quantitative level, human (or interindividual) variability in vulnerability. Considerable progress has been made in understanding the myriad factors that in□uence the magnitude of an individual's external dose of a toxicant, the association between the external dose and the internal (or absorbed) dose (i.e., toxicokinetics), and the biological response at the critical target organs to the internal dose (i.e., toxicodynamics). Epidemiological studies designed to identify susceptibility often succeed—the goal is quite achievable. The Environmental Protection Agency should incorporate those□ndings into quantitative risk assessment now and encourage research that will allow the approach to be extended to more pollutants. The distribution of these important factors is not random within the population. Rather, they co-occur in patterns that result in some subgroups of the population bearing a disproportionate burden of the morbidities caused by toxicants. j

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Drinking Water Infrastructure and Environmental Disparities: Evidence and Methodological Considerations

Potable drinking water is essential to public health; however, few studies have investigated income or racial disparities in water infrastructure or drinking water quality.

There were many case reports documenting a lack of piped water or serious water quality problems in low income and minority communities, including tribal lands, Alaskan Native villages, colonias along the United States–Mexico border, and small communities in agricultural areas.

Only 3 studies compared the demographic characteristics of communities by the quality of their drinking water, and the results were mixed in these studies. Further assessments were hampered by difficulties linking specific water systems to the sociodemographic characteristics of communities, as well as little information about how well water systems operated and the effectiveness of governmental oversight. (Am J Public Health. 2011; 101:S109–S114.doi:10.2105/AJPH.2011.300189)

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WATER SUPPLY INFRASTRUCTURE in the United States ranges from large systems serving millions of people to private wells serving a single family. In all, this infrastructure provides piped water to the homes of over 99% of the US population. Despite such high levels of access, there were reports from several parts of the country suggesting race and income driven disparities in access to piped and/or potable water.[1–6] The extent of disparities in the US drinking water infrastructure and drinking water quality, particularly as related to race and income, has not been well examined. An earlier review of the evidence linking income and race to health risk and drinking water quality identified only a few case studies, concluding "...inequities in exposure to contaminants in water may exist."[7] Seventeen years after this review, only a handful of published studies addressed this issue.

Racial and income disparities in drinking water infrastructure were reviewed with the goal of identifying disparity prone aspects of this infrastructure. As a first step, a framework was proposed that depicted key elements of the drinking water infrastructure in the United States. This framework took a systems approach, thus facilitating identification of aspects of the system that could trigger or enabled disparities, or even limited the mitigation of known disparities. Evidence of infrastructure and concomitant water quality disparities were reviewed using this framework, and the methodological issues that limited the assessment of disparities in water infrastructure were discussed.

FRAMEWORK FOR ASSESSING DISPARITIES

There are many dimensions to the value that consumers ascribe to their water supply: good taste and freedom from odor, low or acceptable health risks, low monetary cost and high convenience, adequate amounts and pressure, high reliability, and reliable information about the quality.[8–11] Disparities in these beneficial characteristics ultimately reflect disparities in the underlying infrastructure. Efforts to reduce these disparities require in-depth understanding of what is disparity prone about this infrastructure; thus, a clear understanding of the elements of a drinking water infrastructure is needed.

The infrastructure that produces water is conceptualized as 4 components: (1) available water sources, (2) the physical infrastructure (e.g., treatment facilities, transmission, and storage), (3) operational/managerial capacity, and (4) government policies and agencies that regulate, assist, and financially support system operators (Figure 1).

Source water quality, location, and reserves drive the technical requirements for water treatment, transmission, and storage. Operation of this system to reliably produce drinking water that meets public health standards at reasonable cost requires adequately trained operators and sufficient administrative capacity to ensure sustainable financial and operational performance. Government serves many roles in this infrastructure: setting policies for water quality regulations and access to sources of water; providing oversight to assure that systems meet water quality, treatment, and monitoring requirements; offering technical assistance and training; and allocating resources to repair and upgrade physical infrastructure.

Particulate Matter–Induced Health Effects: Who Is Susceptible?

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Background: Epidemiological, controlled human exposure, and toxicological studies have

of these studies in a variety of health effects in response to particulate matter (PM) exposure that vary affected.

Objective: To identify populations potentially at greatest risk for PM-related health effects, we evaluated epidemiological studies that examined various characteristics that may influence susceptibility, while using results from controlled human exposure and toxicological studies as supporting evidence. Additionally, we formulated a definition of susceptibility, building from the varied and inconsistent definitions of susceptibility and vulnerability used throughout the literature.

Data synthesis: We evaluated recent epidemiological studies to identify characteristics of populations potentially susceptible to PM-related health effects. Additionally, we evaluated controlled human exposure and toxicological studies to provide supporting evidence. We conducted a comprehensive review of epidemiological studies that presented stratified results (e.g., < 65 vs. ≥ 65 years of age), controlled human exposure studies that examined individuals with underlying disease, and toxicological studies that used animal models of disease. We evaluated results for consistency across studies, coherence across disciplines, and biological plausibility to assess the potential for increased susceptibility to PM-related health effects in a specific population or lifestage.

Conclusions: We identified a diverse group of characteristics that can lead to increased risk of PM-related health effects, including life stage (i.e., children and older adults), preexisting cardiovascular or respiratory diseases, genetic polymorphisms, and low socioeconomic status. In addition, we crafted a comprehensive definition of susceptibility that can be used to encompass all populations potentially at increased risk of adverse health effects as a consequence of exposure to an air pollutant.

key words: children, genetics, lifestyle, minorities, outdoor air, particulate matter, susceptible populations. ***Environ Health Perspect*** **119:446–454 (2011). doi:10.1289/ehp.1002255 [Online 20 October 2010]**

To examine whether particulate matter (PM) differentially affects certain populations, epidemiological studies often conduct stratified analyses, where a greater association between PM and the health effect being examined in one subgroup compared with another provides evidence for a population that may be more susceptible to PM-related health effects. Additionally, controlled human exposure and toxicological studies can provide supporting evidence through the examination of individuals with underlying disease and animal models of disease, respectively. Often the terms "susceptible" and "vulnerable" have been used to characterize these subgroups; however, inconsistency and overlap in these definitions complicate the identification of populations that may be at greatest risk.

In this review, we integrate the evidence from recent epidemiological studies with supporting evidence from controlled human exposure and toxicological studies to identify the characteristics of populations susceptible to PM-related health effects. This review is not intended to be an exhaustive overview of the recent PM literature, but instead a comprehensive evaluation of studies that examined characteristics of potentially susceptible populations.

Defining Susceptibility

The concept of susceptibility is derived from the interindividual variation in human responses to air pollutants, resulting in some populations being at increased risk for air-pollutant–related health effects (Kleeberger and Ohtsuka 2005). "Susceptibility" and "vulnerability" have often been used as distinct terms for identifying these populations, with "susceptibility" referring to biological or intrinsic factors (e.g., life stage, sex) and "vulnerability" referring to nonbiological or extrinsic factors [e.g., socioeconomic status (SES), differential exposure]. However, their definitions vary across reports and studies. We provide some examples below.

- American Lung Association (2001). *Susceptible:* greater likelihood of an adverse outcome given a specific exposure, compared with the general population; includes both host and environmental factors (e.g., genetics, diet, physiologic state, age, and sex, social, economic, and geographic attributes). *Vulnerable:* periods during an individual's life when they are more susceptible to environmental exposures.
- Kleeberger and Ohtsuka (2005). *Susceptible:* intrinsic [e.g., age, sex, preexisting disease (e.g., asthma) and genetics] and extrinsic (e.g., previous exposure and nutritional status) factors.
- Pope and Dockery (2006). *Susceptible:* characteristics that contribute to increased risk of PM-related health effects (e.g., genetics, preexisting disease, age, sex, race, SES, healthcare availability, educational attainment, and housing characteristics).
- Porta (2008). *Susceptible:* vulnerability; lack of resistance to disease; the dynamic state of being more likely or liable to be harmed by a health determinant. *Vulnerable:* a position of relative disadvantage, for example, because of impaired nutrition, cognition, or social position. The extent to which a person, population, or ecosystem is unable or unlikely to respond to threats; may be used as a synonym for "susceptibility."

In addition, the terms "at-risk population" and "sensitive population" have been used in the literature to encompass these concepts more generally.

In many instances, a characteristic that increases a population's risk for morbidity or mortality due to exposure to an air pollutant (e.g., PM) cannot be easily categorized as solely a susceptibility or vulnerability factor due to their overlapping nature, which contributes to the complexity surrounding these concepts. Thus, we developed an all-encompassing definition for the term "susceptible population" as it relates to PM: individual- and population-level characteristics that increase the risk of PM-related health effects in a population, including, but not limited to, genetic background, birth outcomes (e.g., low birth weight, birth defects), race, sex, life stage, lifestyle (e.g., smoking status, nutrition), preexisting disease, SES

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(e.g., educational attainment, reduced access to health care), and characteristics that may modify exposure to PM (e.g., time spent outdoors). Rather than focusing on whether a population is susceptible or vulnerable, we focus instead on the relevant question: which individual- and population-level characteristics result in increased risk of PM-related health effects?

Study Selection

To identify potentially susceptible populations, we focused on the collective evidence evaluated in the most recent science review of the PM National Ambient Air Quality Standards (NAAQS) [U.S. Environmental Protection Agency (EPA) 2009], building upon the evidence presented in previous PM NAAQS reviews (e.g., U.S. EPA 2004). The studies considered [see Supplemental Material, Table 1 (doi:10.1289/ehp.1002255)] examined the health effects due to short-term exposure (i.e., hours to multiple days) and long-term exposure (i.e., months to years) to either both the fine PM fraction [aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$)] and coarse PM fraction [aerodynamic diameter between 10 and 2.5 μm ($PM_{10–2.5}$)] or only one size fraction. Studies that focused on exposure to the thoracic PM fraction [aerodynamic diameter ≤ 10 μm (PM_{10})] are also discussed to the extent that they are informative regarding health effects related to $PM_{2.5}$ and $PM_{10–2.5}$ exposures.

We focused on epidemiological studies that presented stratified results (e.g., males vs. females or < 65 vs. ≥ 65 years of age) because this allowed us to compare populations exposed to similar PM concentrations within the same study design. Results from epidemiological studies provided the basis for characterizing populations potentially susceptible to PM-related health effects. We recognize that epidemiological studies that focus on only one potentially susceptible population (e.g., individuals ≥ 65 years of age or children) may provide supporting evidence on whether a population is susceptible to PM-related health effects, but we do not discuss these studies in this review because of the lack of a comparison population.

We also evaluated controlled human exposure studies that examined individuals with a preexisting disease, and toxicological studies that used animal models of disease. We used these studies to determine whether there was coherence of effects across the scientific disciplines, and to examine biological plausibility for the characteristics identified in epidemiological studies that may confer susceptibility to PM-related health effects. This approach allowed us to evaluate controlled human exposure and toxicological studies that either included or did not include a comparison population. Collectively, the results from stratified analyses in epidemiological studies along with supporting evidence from controlled human exposure and toxicological studies form the overall weight of evidence that we used to assess whether specific characteristics result in a population being susceptible to PM-related health effects.

Life Stage

Occurrence of disease is a reflection of the interaction between host and environmental factors, which varies over time (American Lung Association 2001). Specific populations, particularly children and older adults, are identified as potentially more susceptible than the general population to PM-induced effects as a result of physiological differences.

Children. Children exposed to comparable levels of PM are potentially more susceptible than are adults because of greater time spent outdoors, activity levels, and minute volume per unit body weight, all of which can lead to an increased PM dose per lung surface area and adverse effects on the developing lungs (U.S. EPA 2004). Recent epidemiological studies examined the association between PM and childhood respiratory effects. Collectively, evidence supports increased respiratory effects (e.g., wheeze, cough, respiratory hospital admissions) from short-term PM exposure of all size fractions in children (i.e., < 18 years) compared with adults (e.g., Host et al. 2007; Peel et al. 2005).

Toxicological studies provide support for a biologically plausible mechanism for the increased risk of respiratory effects in children. Altered lung development (i.e., structure and function) was observed in mice chronically exposed to ambient urban air during prenatal and postneonatal periods (Mauad et al. 2008). Additionally, a study demonstrated that exposure of neonatal rats to iron-soot PM resulted in reduced cellular proliferation in certain regions of the lung (e.g., Pinkerton et al. 2008). Together these studies suggest that exposure to PM during critical developmental periods may result in impaired growth of the respiratory system.

Older adults. Older adults are generally considered a susceptible population because of the gradual decline in physiological processes over time (U.S. EPA 2006). For example, dosimetric studies show reduced clearance of PM in all regions of the respiratory tract with increasing age beyond young adulthood (U.S. EPA 2009). Older adults also represent a potentially susceptible population compared with children or younger adults because of the higher prevalence of preexisting cardiovascular and respiratory diseases, which may also confer susceptibility to PM.

Epidemiological evidence indicates increased risk of cardiovascular morbidity with short-term PM exposure in older adults. Several studies reported increased cardiovascular disease (CVD) hospital admissions among older adults compared with all ages or ages < 65 years when exposed to $PM_{2.5}$ (e.g., Pope et al. 2008), $PM_{10–2.5}$ (e.g., Host et al. 2007), and PM_{10} (e.g., Larrieu et al. 2007). However, some studies also revealed no evidence for increased risk of cardiovascular-related hospital admissions among older adults compared with younger ages for $PM_{2.5}$ (e.g., Metzger et al. 2004) or PM_{10} (e.g., Zanobetti and Schwartz 2005). Studies that have examined respiratory-related effects among older adults have not consistently shown associations with PM exposure, but some have reported an increase in respiratory-related hospital admissions (e.g., Fung et al. 2005).

Although the results from the epidemiological literature are mixed regarding morbidity effects from PM exposure, the evidence from controlled human exposure and toxicological studies provides biological plausibility for PM-related cardiovascular effects in older adults. Controlled human exposure studies revealed decreased heart rate variability (HRV) in older adults with or without chronic obstructive pulmonary disease (COPD) after $PM_{2.5}$ concentrated ambient particle (CAPs) exposure (Devlin et al. 2003; Gong et al. 2004a). Using an animal model of terminal senescence, Tankersley et al. (2008) demonstrated altered baseline autonomic tone, reductions in cardiac fractional shortening, and pulmonary vascular congestion after carbon black exposure. Additionally, arrhythmias have been observed in older, but not younger, rats exposed to $PM_{2.5}$ CAPs (Nadziejko et al. 2004).

The continuum of effects from subclinical to cardiovascular- or respiratory-related hospitalization and ultimately death is supported by epidemiological studies showing that older adults (i.e., ≥ 75 years of age in these studies) are more susceptible to nonaccidental mortality upon short-term exposure to $PM_{2.5}$ (e.g., Franklin et al. 2007) and PM_{10} (e.g., Zeka et al. 2006b) compared with younger ages (i.e., < 75 years of age). Similar results were observed in long-term $PM_{2.5}$ exposure studies (e.g., Naess et al. 2007).

Sex

Evidence is not consistent for a difference in PM-related health effects by sex. However, results from dosimetric studies demonstrate sex-related differences in the localization of particles when deposited in the respiratory tract and in the deposition rate due to differences in body size, conductive airway size, and ventilatory parameters (U.S. EPA 2004). Specifically, females have proportionally smaller airways and slightly greater airway reactivity than do males (Yunginger et al. 1992).

Relatively few epidemiological studies (i.e., reviewed in U.S. EPA 2009) have conducted sex-stratified analyses, and these results are

not consistent with the findings of dosimetric studies. When examining the association between short- and long-term $PM_{2.5}$ exposure and cause-specific mortality, existing evidence suggests slightly increased risk for females for nonaccidental mortality (Franklin et al. 2007; Ostro et al. 2006), cardiovascular-related mortality (Chen et al. 2005; Franklin et al. 2007), and lung cancer mortality (Naess et al. 2007), whereas males were at increased risk for respiratory-related mortality (Franklin et al. 2007; Naess et al. 2007). Similarly, associations between short-term exposure to $PM_{10–2.5}$ and nonaccidental and cardiovascular mortality were stronger among females than among males (Malig and Ostro 2009). Collectively, the PM_{10} results (e.g., Chen et al. 2005; Middleton et al. 2008; Wellenius et al. 2006b; Zanobetti and Schwartz 2005; Zeka et al. 2006b) do not support the associations observed in the $PM_{2.5}$ and $PM_{10–2.5}$ studies. For example, slightly stronger associations between PM_{10} and cardiovascular hospital admissions were observed among males than among females (Middleton et al. 2008; Zanobetti and Schwartz 2005), and stronger associations between PM_{10} and respiratory hospital admissions (Middleton et al. 2008) and respiratory mortality (Zeka et al. 2006b) were observed among females than among males. Although human clinical studies are not typically powered to detect differences in response between males and females, one study reported significantly greater decreases in blood monocytes, basophils, and eosinophils in females than in males after controlled exposures to ultrafine (UF) elemental carbon, suggesting potential sex-related differences in subclinical responses upon PM exposure (Frampton et al. 2006).

Race/Ethnicity

Findings from recent epidemiological studies provide evidence that suggests differential susceptibility to PM-induced health effects across races and ethnicities; however, results varied across study locations. The examination of short-term $PM_{2.5}$ exposures and mortality in nine California counties demonstrated an increased risk of mortality for whites and Hispanics but not for blacks (Ostro et al. 2006). An additional analysis in six California counties of associations with $PM_{2.5}$ and various $PM_{2.5}$ components showed increased risk of mortality, specifically cardiovascular mortality, in individuals of Hispanic ethnicity compared with whites (Ostro et al. 2008). In a study in 15 California counties, Hispanics were also found to be at increased risk of cardiovascular mortality with short-term $PM_{10–2.5}$ exposures, but not nonaccidental mortality, compared with whites (Malig and Ostro 2009). Epidemiological studies that examined health effects associated with PM_{10} exposure did not examine Hispanic ethnicity or provide clear evidence for increased risk in a specific race. For example, Zanobetti et al. (2008) found evidence for increased risk of death in other races (i.e., all races except white) compared with whites in a cohort of individuals with COPD in 34 U.S. cities. However, additional multicity studies revealed no evidence for increased risk of congestive heart failure (CHF) hospital admissions (Wellenius et al. 2006b) or cause-specific mortality (Zeka et al. 2006b) when comparing white with other races or blacks, respectively, with short-term PM_{10} exposure.

Genetic Factors

Of recent interest is the potential for gene–environment interactions to affect the relationship between ambient air pollution and the development of health effects (Kauffmann et al. 2004). Numerous studies evaluated the effect of genetic polymorphisms on responses to air pollution exposures in both animals and humans. Functionally relevant polymorphisms in genes can result in a change in the amount or function of the protein product of that gene. Investigations of gene–environment interactions often target polymorphisms in already identified candidate susceptibility genes or in genes whose protein products are thought to be involved in the biological mechanism underlying the adverse effect of an air pollutant. Findings from these studies can provide insight into mechanisms that confer susceptibility to PM-related health effects.

Given evidence that cardiovascular and respiratory effects associated with short-term PM exposure are mediated by oxidative stress (U.S. EPA 2009), new research has focused on the glutathione *S*-transferase (GST) genes, which have common, functionally important polymorphic alleles that significantly affect antioxidant function in the lung (Schwartz et al. 2005). Individuals with genotypes that result in reduced or absent enzymatic activity are likely to have reduced antioxidant defenses and potentially increased susceptibility to inhaled oxidants and free radicals. Because most populations have a high frequency of polymorphisms in the GST genes, individuals with these polymorphisms represent a potentially large susceptible population (Gilliland et al. 2004). Studies of the Normative Aging Study cohort showed that individuals with null GST mu 1 gene (*GSTM1*) alleles had a larger decrease in HRV upon short-term $PM_{2.5}$ exposure than did individuals with at least one functional allele (Chahine et al. 2007; Schwartz et al. 2005). Further, diabetic individuals with null compared with functional *GSTM1* alleles had larger decrements in flow-mediated dilation (FMD), suggesting alterations in endothelial function (Schneider et al. 2008). A controlled human exposure study investigated the effect of allergens and diesel exhaust (DE) particles in individuals with either null genotypes for the GST genes [*GSTM1* and the GST theta-1 gene (*GSTT1*)] or single-nucleotide polymorphisms (SNPs) in the GST pi 1 gene (*GSTP1*; i.e., codon 105 variants), which are hypothesized key regulators of the adjuvant effects of DE on allergic responses (Gilliland et al. 2004). The common *GSTP1* 105 variant (i.e., A105G) results in an amino acid change from isoleucine to valine in the GSTP1 protein and pleiotropic effects on enzymatic function (Gilliland et al. 2004). Gilliland et al. (2004) demonstrated that individuals with the *GSTM1* null or the *GSTP1* I105 wild-type genotypes were more susceptible to allergic inflammation upon exposure to allergen and DE particles than were individuals with functional *GSTM1* and *GSTP1* V105 variant. These results provide evidence of a protective effect with a *GSTP1* polymorphism.

Interactions between GST genes and PM exposure were recently considered in studies of birth outcomes. An epidemiological study examined the association between high PM_{10} exposures (i.e., PM_{10} concentrations ≥ 75th percentile of the PM_{10} distribution) during the third trimester of pregnancy and preterm delivery (Suh et al. 2008). Results showed that women with the *GSTM1* null genotype were at increased risk of preterm birth compared with women who had the functional genotype. Additionally, examination of the statistical interaction between high PM_{10} concentrations during the third trimester of pregnancy and the presence of the *GSTM1* null genotype provided evidence of a synergistic effect on the risk of preterm delivery.

Another gene involved in antioxidant responses, heme oxygenase (decycling) 1 (*HMOX1*), has been examined in a recent panel study. Chahine et al. (2007) found that HRV decreased upon short-term $PM_{2.5}$ exposure in individuals with the long GT tandem repeat polymorphism of the *HMOX1* promoter, and not in individuals with the short repeat variant. This polymorphism is thought to decrease the inducibility of *HMOX1*, whose protein product is heme oxygenase-1, an important antioxidant enzyme (Chahine et al. 2007). Furthermore, when examining a three-way interaction, the effects of $PM_{2.5}$ exposure on HRV were more pronounced in individuals with both the long-repeat *HMOX1* polymorphism and the null *GSTM1* genotype (Chahine et al. 2007).

Additional genes have been examined to determine if specific polymorphisms increase susceptibility to PM-related health effects. A study of the Normative Aging Study cohort focused on polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*) at codon C677T (i.e., CT/TT *MTHFR* genotypes) or the cytoplasmic serine hydroxymethyltransferase gene (*cSHMT*) at codon C1420T (i.e., CT/TT *cSHMT* genotypes) (Baccarelli et al. 2008). The enzymes coded by these genes are involved in folate metabolism and regulate

plasma homocysteine levels, which is a risk factor for CVD. The CT/TT *MTHFR* variants are linked to reduced enzymatic activity, whereas it is unclear whether this is the case for the CT/TT *cSHMT* variants (Lim et al. 2005). Additionally, *MTHFR* and *cSHMT* were found to interact such that the effect of the *MTHFR* polymorphism on the risk of CVD varied by the *cSHMT* genotype (Lim et al 2005). Baccarelli et al. (2008) found that baseline HRV was lower in individuals with the CT/TT *MTHFR* genotypes than in individuals with the CC genotype, but they observed no relationship between HRV and *cSHMT* genotypes. However, the association between HRV and $PM_{2.5}$ exposure was modulated by both *MTHFR* and *cSHMT*. Specifically, Baccarelli et al. (2008) observed a larger HRV reduction upon $PM_{2.5}$ exposure in individuals with CT/TT *MTHFR* genotypes or the CC *cSHMT* genotype compared with the CC *MTHFR* genotype or CT/TT *cSHMT* genotypes. These results suggest a protective effect conferred by certain gene variants of *MTHFR* and *cSHMT* on PM-mediated alterations in HRV.

Investigations of polymorphisms of the fibrinogen genes (*FGA* and *FGB*) have also been conducted. Peters et al. (2009) examined the effect of SNPs in *FGA* and *FGB* on steady-state levels of plasma fibrinogen. Because fibrinogen has been implicated in atherothrombosis, it is thought to play a role in PM-mediated CVD. In a population of myocardial infarction (MI) survivors, an increase in plasma fibrinogen levels upon PM_{10} exposure was 8-fold higher in individuals with one homozygous minor allele genotype than in individuals homozygous for the major allele of *FGB*. Therefore, the combination of inflammatory effects and higher fibrinogen levels attributed to PM exposure in individuals with certain polymorphisms could increase the risk of PM-related cardiovascular effects (Peters et al. 2009).

Collectively, these results suggest that the presence of null alleles or specific polymorphisms in genes that mediate the antioxidant response, regulate folate metabolism, or regulate levels of fibrinogen may increase susceptibility to PM-related health effects. However, in some cases genetic polymorphisms may confer protective effects, such as those demonstrated for certain *GSTP1* variants. Thus, genetic factors can modulate the relationship between ambient PM exposure and the development of health effects by either increasing or decreasing the risk of a cardiovascular or respiratory outcome.

Obesity

Pulmonary oxidative stress resulting from inhaled PM may lead to systemic inflammation and, subsequently, increased cardiovascular risk (Dubowsky et al. 2006). As a result, studies have recently focused on chronic inflammatory conditions, such as obesity, that may modulate PM-related health effects. From 1960 to 2004, the prevalence of overweight [body mass index (BMI) ≥ 25.0 kg/m^2)] and obese (BMI ≥ 30.0 kg/m^2) individuals in the United States increased from 20% to 74% and 13.3% to 32.1%, respectively (National Center for Health Statistics 2006).

Numerous studies have examined whether individuals who are overweight or obese are at increased risk of adverse health effects of PM relative to people of normal weight. Epidemiological studies reported a reduction in HRV in obese compared with nonobese subjects upon PM exposure (e.g., Schwartz et al. 2005). Additionally, studies observed higher levels of inflammatory markers in the plasma [i.e., C-reactive protein (CRP), interleukin-6 (IL-6), and white blood cell (WBC) count] (Dubowsky et al. 2006) and evidence for a larger reduction in FMD (Schneider etal. 2008) in obese than in nonobese individuals in response to short-term $PM_{2.5}$ exposure. Studies of the Veteran's Normative Aging and Women's Health Initiative cohorts provided evidence for an increase in inflammatory markers and cardiovascular events, respectively, upon long-term PM exposure in individuals with BMI ≥ 25 kg/m^2 compared with < 25 kg/ m^2 (Miller et al. 2007; Zeka et al. 2006a). However, an examination of associations between 20 -year exposures to PM_{10} or $PM_{2.5}$ and subclinical atherosclerosis in the Multi-ethnic Study of Atherosclerosis cohort provided no clear evidence for differences by BMI (i.e., > 30 kg/m^2 vs. < 30 kg/m^2) (Diez Roux et al. 2008). The greater response observed in obese individuals to PM exposure could be due, in part, to a higher PM dose rate in obese individuals. This has been demonstrated in overweight children, where an increase in tidal volume and resting minute ventilation was observed with higher BMI (Bennett and Zeman 2004).

Preexisting Diseases

The National Research Council (2004) has emphasized the need to evaluate the effect of air pollution on potentially susceptible populations, including those with cardiovascular and respiratory diseases. Previous reviews of the literature suggested that preexisting cardiopulmonary diseases, as well as diabetes, may increase susceptibility to effects of PM exposure (U.S. EPA 2004). More recent epidemiological and experimental studies have built upon these conclusions to provide an additional understanding of susceptibility to PM-related health effects.

Cardiovascular disease. Epidemiological, controlled human exposure, and toxicological studies examined whether hypertension, conditions associated with coronary artery disease [CAD; i.e., ischemic heart disease (IHD), MI, atherosclerosis], and CHF modulate PM-related health effects. Preexisting cardiovascular conditions, such as hypertension, heart disease, and coronary heart disease, are highly prevalent in the U.S. population (Table1).

Hypertension. Hypertension has often been considered in stratified analyses that examine the association between short-term PM exposure and cardiovascular-related

Table 1. Percentages of the U.S. population with CVD, respiratory diseases, and diabetes.

	Adults (≥ 18)		Age (years)				Region			
Chronic condition/disease	*n* (× 10^6)	(%)	18–44	45–64	65–74	≥ 75	NE	MW	S	W
CVD										
All heart disease[a]	25.1	11.2	4.1	12.2	27.1	35.8	10.6	12.3	11.3	10.2
Coronary heart disease[b]	13.7	6.1	0.9	6.7	18.6	23.6	5.3	6.7	6.4	5.5
Hypertension	51.6	23.2	8.2	32.1	50.9	57.4	21.3	23.4	25.1	21.0
Stroke	5.4	2.4	0.3	2.8	6.3	10.6	2.2	2.3	2.7	2.2
Respiratory diseases										
Asthma[c]	24.2	11.0	11.5	10.5	11.7	9.3	11.7	11.5	10.5	10.8
COPD										
Chronic bronchitis	7.6	3.4	2.3	4.2	5.5	4.8	2.8	3.2	4.0	2.9
Emphysema	3.7	1.6	0.2	2.3	4.5	5.2	1.1	1.8	1.8	1.6
Diabetes	17.2	7.7	2.2	10.6	19.9	17.2	6.3	7.7	8.3	7.6

Abbreviations: NE, Northeast; MW, Midwest; S, South; W, West. All data are from the Centers for the Disease Control and Prevention (2008a, 2008b).
[a]Heart disease includes coronary heart disease, angina pectoris, heart attack, or any other heart condition or disease. [b]Coronary heart disease includes coronary heart disease, angina pectoris, or heart attack. [c]Prevalence data are based on adults responding to "ever told had asthma."

hospital admissions and emergency department (ED) visits. However, it is unclear whether preexisting hypertension modifies the associations observed. A study conducted in Utah found no evidence for increased risk of acute IHD events for $PM_{2.5}$ exposure in individuals with preexisting hypertension compared with those without hypertension (Pope et al. 2006). This result is consistent with other studies where hypertension did not modify the association between PM and cardiovascular outcomes, such as CHF hospital admissions (e.g., Wellenius et al. 2006b). In contrast, Peel et al. (2007) found that the presence of preexisting hypertension resulted in an increased risk of ED visits for dysrhythmias and CHF with PM_{10} exposure. The potential effect of hypertension on the manifestation of PM-related cardiovascular effects is supported by a toxicological study conducted in a rat model of hypertension, which demonstrated that $PM_{2.5}$ CAPs exposure resulted in higher mean arterial pressure compared with air controls (Sun et al. 2008). This finding suggests a relationship between $PM_{2.5}$ exposure and hypertension that may provide biological plausibility for the worsening of hypertension-related cardiovascular outcomes observed by Peel et al. (2007).

CAD. We identified multiple studies that examined the effect of preexisting cardiovascular conditions associated with CAD on PM-related cardiovascular effects. In a panel study in Boston, individuals with preexisting IHD were observed to have larger alterations in HRV with $PM_{2.5}$ exposure than did individuals without IHD (Park et al. 2005). Toxicological studies using Boston CAPs in dogs with induced myocardial ischemia, an animal model that mimics the pathophysiological effects associated with IHD, demonstrated increased ST-segment elevation and impaired myocardial blood flow in response to $PM_{2.5}$ CAPs exposure (Bartoli et al. 2009; Wellenius et al. 2003).

Epidemiological, controlled human exposure, and toxicological studies examined the effect of previous MI on PM-induced cardiovascular effects. Wellenius et al. (2006b) found no evidence to suggest a modification of the relationship between PM_{10} and CHF hospital admissions by previous acute MI. Controlled human exposure studies investigated the effects of dilute DE or fine and UF CAPs in subjects with CAD and prior MI (Mills et al. 2007, 2008). Exposure to fine and UF CAPs, which were low in combustion-derived particles, did not result in any pronounced effects on vascular function (Mills et al. 2008). However, exposure to dilute DE promoted exercise-induced ST-segment changes, which are consistent with myocardial ischemia, and inhibited endogenous fibrinolytic capacity (Mills et al. 2007). The discrepant results in these studies may be due to medication use, because individuals with CAD (most on beta blockers) exposed to UF carbon particles had no change in HRV (Routledge et al. 2006), or due to differences in the PM. In a toxicological study using an animal model of acute MI, rats exposed to $PM_{2.5}$ CAPs had decreased ventricular premature beats and spontaneous supraventricular ectopic beats (Wellenius et al. 2006a). In a rodent MI model of chronic heart failure, a prominent increase in the incidence of premature ventricular contraction with DE exposure was reported (Anselme et al. 2007). The discrepancy in health effects observed between toxicological studies could be due to differences in the MI model or the PM (i.e., CAPs vs. DE).

Toxicological studies also examined the effects of PM exposure in a murine model susceptible to atherosclerosis, the apolipoprotein knockout ($ApoE^{-/-}$) mouse, which is characterized by systemic oxidative stress. $ApoE^{-/-}$ mice acutely exposed to whole gasoline emissions resulted in electrocardiogram T-wave alterations, which were attributable to particles (Campen et al. 2006). Several studies reported relatively consistent pathophysiological effects when exposing $ApoE^{-/-}$ mice to $PM_{2.5}$ CAPs for several months. Chen and Nadziejko (2005) found a greater degree of atherosclerosis in $ApoE^{-/-}$ mice than in control mice after exposure to fine CAPs (from Tuxedo, NY). Furthermore, decreased heart rate, physical activity, and temperature along with biphasic responses in HRV were observed in $ApoE^{-/-}$ mice, but not in control mice, upon exposure to these CAPs (Chen and Hwang 2005). In addition, $ApoE^{-/-}$ mice exposed to UF and $PM_{2.5}$ CAPs (from Los Angeles and Tuxedo) had larger atherosclerotic lesions than those exposed to air (e.g., Araujo et al. 2008; Sun et al. 2008).

Taken together, the results from toxicological studies using models relevant to CAD provide coherence and biological plausibility for the epidemiological findings of PM-related cardiovascular effects.

CHF. A limited number of epidemiological studies have examined potential effect measure modification of PM-related cardiovascular effects by comparing individuals with and without preexisting CHF. In Utah, short-term $PM_{2.5}$ exposure was associated with increased risk of hospital admissions for acute IHD events in individuals with preexisting CHF (Pope et al. 2006). Additionally, a study conducted in Cook County, Illinois, showed that individuals with preexisting CHF were at increased risk of PM-related mortality (Bateson and Schwartz 2004). However, a large multicity study revealed no evidence of increased risk of MI hospital admissions with exposure to PM_{10} in individuals with versus without CHF (Zanobetti and Schwartz 2005).

Respiratory diseases. Epidemiological studies have examined the effect of preexisting respiratory diseases on multiple health outcomes (e.g., asthma symptoms, mortality) in response to PM exposure. In addition, animal models have been developed, and controlled human exposure studies have examined the possible effect of preexisting respiratory conditions on PM-induced health effects in an experimental setting. As was true for CVD millions of people are affected by respiratory diseases (i.e., asthma, COPD, and emphysema) in the United States, which includes approximately 9.3% of children < 18 years of age that have been diagnosed with asthma (see Table 1) (Pleis and Lucas 2009).

Asthma. In epidemiological studies of asthmatic children, short-term $PM_{2.5}$ exposure was associated with an increase in medication use (Rabinovitch et al. 2006) and respiratory symptoms (i.e., cough, shortness of breath, and chest tightness) (e.g., Gent et al. 2003), and short-term PM_{10} exposure was associated with morning symptoms (Mortimer et al. 2002) and respiratory symptoms (Delfino et al. 2003). Health effects in asthmatic adults have also been demonstrated (e.g., asthma attacks with short-term PM_{10} exposure), although the evidence is more limited (Desqueyroux et al. 2002).

Toxicological studies provide coherence and biological plausibility for the findings of the epidemiological literature. In response to an acute exposure to CAPs from Detroit, an area with pediatric asthma rates three times the national average, rats with allergic airway disease exposed to PM derived from local combustion sources had eosinophil influx and increased bronchoalveolar lavage fluid protein content (Morishita et al. 2004). These findings suggest that the presence of allergic airway conditions increases susceptibility to allergic airway responses to $PM_{2.5}$, which may be partially attributed to increased pulmonary deposition and localization of particles in the respiratory tract (Morishita et al. 2004). An additional study using rats with allergic airways disease exposed to CAPs provided evidence for increased expression of genes associated with inflammation and airway remodeling compared with nonallergic animals exposed to CAPs and allergic animals not exposed to CAPs (Heidenfelder et al. 2009). Furthermore, several toxicological studies demonstrated that PM acts as an adjuvant to enhance the severity or development of asthma (e.g., Li et al. 2009).

The results from the epidemiological and toxicological studies that focused on preexisting allergic airways disease are supported by a collection of controlled human exposure studies demonstrating that exposure to DE particles increases the allergic inflammatory response in atopic individuals (e.g., Bastain et al. 2003; Nordenhäll et al. 2001). However,

not all controlled human exposure studies provided evidence for enhanced respiratory effects in asthmatic individuals. For example, a series of studies reported that healthy and asthmatic subjects exposed to CAPs of three different size fractions ($PM_{10–2.5}$, $PM_{2.5}$, and UF) exhibited similar respiratory responses (e.g., Gong et al. 2003, 2004b). However, these studies excluded moderate and severe asthmatics, which would be expected to show increased susceptibility to PM exposure.

COPD. Epidemiological panel studies that examined the effect of PM on lung function demonstrated greater declines in forced expiratory volume in 1 sec and forced vital capacity in individuals with COPD versus those without in response to $PM_{2.5}$ exposure (e.g., Lagorio et al. 2006; Trenga et al. 2006). Conversely, in a study involving controlled human exposures to $PM_{2.5}$ CAPs, healthy older adults experienced a somewhat greater PM-induced respiratory response (decrease in arterial oxygen saturation) than did older adults with COPD (Gong et al. 2004a). No other respiratory effects in response to PM exposure (e.g., respiratory symptoms, lung function, or airway inflammation) were observed in either group.

Dosimetric studies clearly demonstrated that COPD patients have increased dose rates of fine and UF particles and impaired mucociliary clearance relative to age-matched healthy subjects. These findings suggest that individuals with COPD are potentially at greater risk of PM-related health effects (Bennett et al. 1997; Brown et al. 2002). Support for PM-mediated exacerbation of emphysema is provided by a toxicological study using papain-treated mice. In this model, exposure to urban ambient air resulted in a PM-dependent increase in a measure of airspace enlargement (Lopes et al. 2009). The pathogenesis of emphysema is a complex process involving oxidative stress and inflammation, both of which can result from PM deposition in the respiratory tract. Collectively, these results provide preliminary evidence for biological plausibility of PM-related health effects in individuals with COPD and suggest that respiratory morbidities, excluding asthma, may also increase the susceptibility of a population to PM-related respiratory effects.

Respiratory contributions to cardiovascular effects. Most studies that examined whether preexisting respiratory diseases increase the risk of PM-related health effects have focused on PM-induced respiratory exacerbations, but some studies have also examined whether preexisting respiratory diseases contribute to cardiovascular effects. Most epidemiological studies did not find evidence that preexisting respiratory diseases increased the risk of PM-related cardiovascular hospital admission or ED visits for a variety of cardiovascular outcomes (e.g., IHD, arrhythmias, CHF, MI); these studies examined whether preexisting respiratory infection (Wellenius et al. 2006b), pneumonia (Zanobetti and Schwartz 2005), and COPD (Peel et al. 2007) increased the risk of PM-related cardiovascular effects. However, De Leon et al. (2003) found that individuals with preexisting respiratory diseases had increased risk for PM_{10}-induced circulatory mortality compared with individuals without preexisting respiratory diseases.

A controlled human exposure study demonstrated acute responses in the cardiovascular system and systemic circulation among asthmatic individuals, compared with nonasthmatics, after $PM_{2.5}$ CAPs exposure (Gong et al. 2003). However, respiratory disease does not consistently affect cardiovascular response to PM exposure in controlled human exposure studies (e.g., Fakhri et al. 2009; Gong et al. 2004b). A toxicological study showed that the pulmonary artery lumen-to-wall ratio was decreased in an animal model of chronic bronchitis in response to $PM_{2.5}$ CAPs, but a similar response was also observed in healthy rats (Batalha et al. 2002). Whereas the identification of characteristics of potentially susceptible populations has initially relied on epidemiological evidence, in this instance it is unclear how the epidemiological results compare with those found in the controlled human exposure and toxicological studies that focused on exposure to $PM_{2.5}$ (e.g., CAPs). Thus, the lack of coherence across disciplines clouds whether individuals with preexisting respiratory diseases represent a population that is potentially susceptible to PM-related cardiovascular effects.

Diabetes. Numerous studies have evaluated the potential for diabetes, a disease linked to chronic inflammation, to increase the risk of PM-related health effects. The increased interest in this population can be partially attributed to the large percentage of diabetic individuals in the United States (Table 1).

Epidemiological studies that examined whether diabetes modifies the association between cardiovascular effects and PM exposure primarily focused on short-term PM_{10} exposure. A multicity study showed > 75% greater risk of hospitalization for cardiac diseases with PM_{10} exposure among individuals with diabetes than among those without diabetes (Zanobetti and Schwartz 2002). A study conducted in Atlanta, Georgia, also showed increased risk of cardiovascular-related ED visits for PM_{10} exposure, specifically for IHD, arrhythmias, and CHF, among persons with diabetes than among those without diabetes (Peel et al. 2007). However, other studies (both multicity and single city) revealed no evidence for increased risk of cardiovascular ED visits and hospital admissions for short-term $PM_{2.5}$ or PM_{10} exposure among persons with diabetes compared with those without diabetes (Pope et al. 2006; Wellenius et al. 2006b; Zanobetti and Schwartz 2005). Other evidence from epidemiological studies indicates that diabetes could potentially increase the risk of mortality with exposure to $PM_{2.5}$ (Goldberg et al. 2006) and PM_{10} (Zeka et al. 2006b).

Additional epidemiological studies, as well as controlled human exposure studies, examined physiological alterations and changes in inflammatory and coagulation markers in the cardiovascular system of diabetic individuals in an attempt to provide biological plausibility for the increased risk of cardiovascular effects observed in some of the population-level studies. A panel study of individuals with diabetes demonstrated that ambient exposure to $PM_{2.5}$ enhanced the reduction in various markers of endothelial function (Schneider et al. 2008). Liu et al. (2007) observed an increase in alterations in FMD and basal diameter upon PM_{10} exposure in persons with diabetes. On the other hand, a controlled human exposure study showed that DE elicited no prothrombotic effects in subjects with metabolic syndrome, which is characterized by alterations in physiological parameters and inflammatory markers similar to those observed in individuals with diabetes (Carlsten et al. 2008). An examination of biomarkers in individuals with diabetes who were exposed to PM revealed mixed results, including an increase in von Willebrand factor (Liao et al. 2005), an increase in thiobarbituric acid but no increases in CRP or tumor necrosis factor-α (Liu et al. 2007), and an increase in CRP and WBC count (Dubowsky et al. 2006). Although it is unclear how alterations in each of these biomarkers contribute to the potential for cardiovascular effects in individuals with diabetes, PM-induced changes in inflammation, oxidative stress, and acute-phase response may lead to more severe cardiovascular effects.

Socioeconomic Status

In 2009, approximately 14.3% of the U.S. population was living in poverty (U.S. Census 2010). Although there are numerous indicators of SES, including economic status measured by income, social status measured by education, and work status measured by occupation, each of these linked factors can influence a population's susceptibility to PM-related health effects (Dutton and Levine 1989). Low SES is associated with a higher prevalence of preexisting diseases, limited access to medical care, and limited access to fresh foods leading to a reduced intake of polyunsaturated fatty acids and vitamins, all of which may contribute to increased susceptibility to PM-induced health effects (Kan et al. 2008).

Indicators of SES were demonstrated in some epidemiological studies to modify health outcomes associated with PM exposure. In these studies, SES has primarily been defined at the neighborhood level (e.g.,

educational attainment or income within a neighborhood) to identify low, medium, and high SES areas within a study location. Educational attainment generally coincides with an individual's income, which is correlated with other indicators of SES, such as residential environment (Jerrett et al. 2004). Epidemiological studies reported increased risk of mortality for short- term exposure to $PM_{2.5}$ and $PM_{2.5}$ components in low-SES groups (i.e., examined by median household income) (Franklin et al. 2008), whereas other analyses demonstrated consistent trends of increased mortality associations with $PM_{2.5}$, $PM_{2.5}$ species, and $PM_{10–2.5}$ for low educational attainment groups (i.e., ≥ high school vs. < high school education) (Ostro et al. 2006, 2008; Zeka et al. 2006b). In the American Cancer Society cohort, increased lung cancer mortality with long-term $PM_{2.5}$ exposure was observed among the subgroup with a high school education or less compared with groups with more than a high school education (Krewski et al. 2009). However, when examining $PM_{2.5}$-related IHD mortality by education level, the reverse relationship was observed (Krewski et al. 2009).

Epidemiological studies also examined other indicators of SES, such as residential location and nutritional status, to identify their influence on the PM–health effect association. An examination of the potential modification of acute mortality effects due to PM exposure by residential location in Hamilton, Canada, using educational attainment as an indicator for SES revealed that the areas of the city with the highest SES displayed no evidence of effect measure modification, whereas the areas with the lowest SES had the largest mortality risks (Jerrett et al. 2004). Likewise, a study conducted in Phoenix used educational attainment (i.e., percentage of population with less than a high school diploma) and income (i.e., percentage of population with income below the poverty level) to represent SES (Wilson et al. 2007); the area with the lowest SES had the strongest association between $PM_{2.5}$ and cardiovascular mortality, but the association differed when examining $PM_{10–2.5}$, with the strongest association being observed for the area with higher educational attainment and income.

Another consequence of low SES may be decreased access to fresh foods. The effect of nutritional deficiencies was examined in a study of individuals with polymorphisms in genes associated with increased risk of CVD (Baccarelli et al. 2008). Individuals who had these genetic polymorphisms and who increased their intake (above median levels) of B_6, B_{12}, or methionine did not have alterations in HRV in response to $PM_{2.5}$ exposure, in contrast to those individuals who did not increase nutrient intake (Baccarelli et al. 2008).

Table 2. Susceptibility characteristics.

Characteristic	Susceptible population
Life stage	Children (< 18 years of age)
	Older adults (≥ 65 years of age)
Sex	—[a]
Race/ethnicity	—[a]
Genetic factors	Genetic polymorphisms: GST genes, *HMOX1*,[b] *MTHFR*,[b] *cSHMT*,[b] *FGB*[b]
Preexisting diseases	CVD: CAD
	Respiratory diseases: asthma, COPD[b]
	Diabetes[b]
Obesity	Increased BMI[b]
SES	Low educational attainment
	Low income

[a]Of the studies evaluated, current evidence does not indicate one population is more susceptible to PM-related health effects than another. [b]Additional evidence is needed to confirm whether the characteristic evaluated results in increased susceptibility to PM-related health effects.

Conclusion

Epidemiological studies have examined characteristics of populations that may render them more susceptible to PM-related health effects by conducting stratified analyses. By also considering experimental studies that examined individuals with an underlying health condition or used animal models of disease, it is possible to more thoroughly evaluate characteristics that may lead to increased susceptibility. The collective evidence across disciplines indicates that some characteristics, including life stage, genetic polymorphisms, preexisting cardiovascular and respiratory diseases, and SES, may increase the susceptibility of populations to PM-related health effects (Table 2). Additional characteristics (e.g., obesity and diabetes) were also identified.

A limitation of this review, as described throughout, is the inability to clearly state the overall strength of the evidence for some characteristics of potentially susceptible populations because of inconsistency in the evidence across epidemiological studies or lack of information from experimental studies regarding biologically plausible mechanisms. It has been noted, specifically in a recent review involving controlled human exposures to PM among potentially susceptible groups, that the relative lack of evidence of increased susceptibility may be due to a host of factors, such as medication use of the volunteers, subject selection bias, and nonspecificity of study end points, and not necessarily because these individuals did not represent populations susceptible to PM-related health effects (Huang and Ghio 2009). As a result, the collective evidence discussed within this review may not clearly identify all the characteristics of populations susceptible to PM-related health effects.

To assist in the identification of populations at increased risk for PM-related health effects, a consistent definition of susceptibility is needed. The ambiguity in the use of terms, including "susceptibility," "vulnerability," and "sensitivity," across studies has to an extent increased the difficulty in focusing on the populations that have a greater likelihood of experiencing PM-related health effects. In the future, an approach similar to the one used in this review may allow the scientific community to focus on identifying the populations at increased risk to an air pollutant, regardless of their classification (e.g., susceptible, vulnerable, sensitive).

Overall, the epidemiological studies evaluated in this review, with supporting evidence from controlled human exposure and toxicological studies, identified characteristics of populations that may lead to increased susceptibility to PM-related health effects. This includes life stage, specifically children and older adults; preexisting cardiovascular (i.e., CAD) and respiratory (i.e., asthma) diseases; genetic polymorphisms; and low SES, as measured by educational attainment and income. Additionally, more limited evidence suggests an increase in PM-related health effects in individuals with diabetes, COPD, and increased BMI. Although not clearly established, the evidence evaluated also indicated potentially increased risk of PM-related health effects by sex and race/ethnicity, but these associations were not consistent across PM size fractions, health effects, and in some cases study locations. Overall, additional research is warranted to more accurately identify the characteristics of potentially susceptible populations and the biologically plausible mechanisms that result in one population being more susceptible than another to PM -related health effects. In addition, future research may enable the identification of specific PM size fractions, sources, or components that render a population more susceptible.

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C. Committee

Statement of Task

The Standing Committee will examine, explore, and consider issues on the use of emerging science for environmental health decisions. The committee will provide a public venue for communication among government, industry, environmental groups, and the academic community about scientific advances in methods and approaches that can be used in the identification, quantification and control of environmental impacts on human health. The topics covered will explore new developments in the life sciences, bioinformatics, modeling, and risk or decision analyses that could be applicable to environmental health decision making. Specifically, the committee will consider topics that fall within the following four themes:

1) Emerging scientific tools or data that may address existing issues in environmental health
2) Emerging areas of science that have not traditionally been applied to environmental health research and issues
3) Current and pressing environmental health issues for which new science, tools, or data may offer new insights, approaches, or solutions
4) Practical issues facing the environmental health science community as it deals with the emerging science

The Standing Committee will accomplish its task by convening public meetings of invited experts to inform the committee and the sponsor about key scientific issues relevant to the use of emerging scientific information, knowledge, and approaches in regulation, disease prevention, education and personal choice, and clinical intervention and management of diseases caused and/or modified by environmental factors. Participants in the public meetings will include members of government, industry, environmental groups, and the academic community. These public meetings will also be made available to a broader audience via the internet, and highlights of the discussions will be included in regular newsletters prepared by NAS staff.

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NATIONAL INSTITUTES OF HEALTH

National Institute of Environmental Health Sciences

John Balbus, M.D., M.P.H.
(Chair)
Senior Advisor for Public Health
National Institute of Environmental Health Sciences

National Toxicology Program (NTP)

John R. Bucher, Ph.D.
Associate Director, Natural Toxicology Program
National Institutes of Environmental Health Sciences

Extramural Training and Research

David Balshaw
National Institutes of Health

National Institute of Child Health and Human Development (NICHD)

Division of Intramural Population Health Research (DIPHR)
Office of the Director

Germaine Buck Louis, Ph.D.
Director and Senior Investigator
Epidemiology, Statistics and Prevention Research (DESPR),
Epidemiology Branch
National Institutes of Health

National Center for Advancing Translational Sciences

David L. Gerhold, Ph.D.
Leader, Genomic Toxicology
Division of Pre-Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health

UNITED STATES DEPARTMENT OF AGRICULTURE

Food Safety and Inspection Service (FSIS)

Office of Public Health and Science (OPHS)

Kerry L. Dearfield, Ph.D.
Chief Scientist
Office of Public Health Science
Food Safety and Inspection Service
U.S. Department of Agriculture

DEPARTMENT OF THE INTERIOR

U.S. Geological Survey

Ecosystems Mission Area

Sarah Gerould, Ph.D.
Chief of Staff
Ecosystems Mission Area
U.S. Geological Survey

CONSUMER PRODUCT SAFETY COMMISSION

Office of Hazard Identification and Reduction

Treye Thomas, Ph.D.
Toxicologist
Chemical Hazards Program Leader
Office of Hazard Identification and Reduction
U.S. Consumer Product Safety Commission

DEPARTMENT OF DEFENSE

Department of the Army

U.S. Army Center For Environmental Health Research

Pulmonary Health Program

David A. Jackson, Ph.D.
Program Director
Pulmonary Health Program
US Army Center for Environmental Health Research

U.S. Army Public Health Command

Bradley C. Nindl, Ph.D., FACSM
Scientific Advisor
Army Institute of Public Health
Army Public Health Command

Defense Threat Agency

Alison E. Director-Myska, Ph.D.
Senior S&T Manager, J9CBS
Chemical/Biological Technologies
Defense Threat Reduction Agency

Akbar S. Khan, Ph.D.
Microbiologist & Program Manager
Defense Threat Reduction Agency

Office of the Secretary of Defense (OSD)

Office of the Deputy Assistant Secretary of Defense

Steve Jones, MPH
Program Director, Force Readiness and Health Assurance
Policy ODASD(HA)-Health Readiness Policy & Oversight

Department of Labor

Occupational Safety and Health Administration (OSHA)

Amanda Edens, M.P.H.
U.S Department of Labor, Occupational Safety and Health Administration
Directorate of Standards and Guidance

NATIONAL AERONAUTICS & SPACE ADMINISTRATION

Environmental Management Division

Linda S. Wennerberg, Ph.D.
NASA HQ
Environmental Management Division

William H. Farland (Chair), PhD, ATS, is the Senior Advisor to the Executive Vice President, Colorado State University and a professor in the Department of Environmental and Radiological Health Sciences, School of Veterinary Medicine and Biomedical Sciences. Formerly, Dr. Farland served as Vice President for Research from 10/2006-9/2013. Dr. Farland holds a Ph.D. (1976) from UCLA in cell biology and biochemistry. In 2006, Dr. Farland was appointed Deputy Assistant Administrator for Science in the U.S. Environmental Protection Agency's Office of Research and Development (ORD). He had served as the Acting Deputy Assistant Administrator since 2001. In 2003, Dr. Farland was also appointed Chief Scientist in the Office of the Agency Science Advisor. He served as the EPA's Acting Science Advisor throughout 2005. Prior to that, he was the Director of the ORD's National Center for Environmental Assessment. Dr. Farland's 27 year federal career was characterized by a commitment to the development of national and international approaches to the testing and assessment of the fate and effects of environmental agents. Dr. Farland has continually served on a number of executive-level committees and advisory boards within the Federal government. In 2005-2006, he chaired the Executive Committee of the National Toxicology Program (NTP). He was also a member of the Scientific Advisory Council of the Risk Sciences and Public Policy Institute, Johns Hopkins University School of Hygiene and Public Health; a public member of the American Chemistry Council's Strategic Science Team for its Long-Range Research Initiative, and a member of the Programme Advisory Committee for the WHO's International Programme on Chemical Safety. Dr. Farland served as Chair of an External Advisory Group for the National Institute of Environmental Health Sciences (NIEHS) regarding the future of the Superfund Basic Research Program. In 2013, Dr. Farland was appointed to the Board on Environmental Studies and Toxicology (BEST) of National Research Council (NRC). He also chairs a standing committee on Emerging Science for Environmental Health Decisions of the NRC and was a member of a NRC Committee to Develop a Research Strategy for Environmental, Health, and Safety Aspects of Engineered Nanomaterials. In 2002, Dr. Farland was recognized by the Society for Risk Analysis with the "Outstanding Risk Practitioner Award," and in 2005 was appointed as a Fellow of the Society. In 2006, he received a Presidential Rank Award for his service as a federal senior executive. In 2007, he was elected as a Fellow, Academy of Toxicological Sciences. Dr. Farland continues to teach, publish and serve as a reviewer in environmental toxicology and risk assessment.

George P. Daston, PhD, has been employed at Procter & Gamble Company since 1985, where he is Victor Mills Society Research Fellow. Dr. Daston has spent his entire career in research to understand the effects of exogenous chemicals on biological systems, especially the developing embryo, fetus and child. His research interests include teratogenic mechanisms, in vitro methodologies, and risk assessment. He has published over 100 peer-reviewed articles, reviews and book chapters, and has edited three books. Dr. Daston's professional activities include serving as Councilor of the Society of

Toxicology (2001-03); President (1999-2000) of the Teratology Society; member of the National Academy of Sciences Board on Environmental Studies and Toxicology (1995-98); member of the EPA Board of Scientific Counselors (2002-08); member of the U.S. National Toxicology Program Board of Scientific Counselors (2003-06, Chair in 2006); member of the National Children's Study Advisory Committee (2003-06); and member of EPA's Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC). He has served on several NRC committees, including the Committee on Developmental Toxicology, Committee on Research Opportunities and Priorities for EPA, and the Subcommittee on Arsenic in Drinking Water. Dr. Daston has served on the organizing committees for numerous government and private sector-organized workshops on reproductive toxicity, risk assessment, and non-animal alternatives. He chaired NIEHS/ICCVAM working groups evaluating the state of validation of the Frog Embryo Teratogenesis Assay – Xenopus (FETAX) assay for teratogen screening and receptor binding and transcriptional activation assays for estrogens and androgens. Dr. Daston is Editor-in-Chief of Birth Defects Research: Developmental and Reproductive Toxicology. Dr. Daston is an Adjunct Professor in the Department of Pediatrics and Developmental Biology Program at the University of Cincinnati and Children's Hospital Research Foundation. Dr. Daston received his Ph.D. from the University of Miami and post-doctoral training at the U.S. EPA's laboratories in Research Triangle Park, North Carolina.

Richard A. Denison, PhD, is a lead senior scientist at the Environmental Defense Fund. Dr. Denison has 30 years of experience in the environmental arena, specializing in chemicals policy and hazard, exposure, and risk assessment and management for industrial chemicals and nanomaterials. Dr. Denison is a member of the National Academy of Sciences' Standing Committee on Emerging Science for Environmental Health Decisions. Until recently, he was on the NAS Board on Environmental Studies and Toxicology and served on the Green Ribbon Science Panel for California's Green Chemistry Initiative. Dr. Denison has testified before various Congressional committees on the need for fundamental reform of US policy toward industrial chemicals and on nanomaterial safety research needs. He served as a member of the National Academy of Sciences' Committee to Develop a Research Strategy for Environmental, Health and Safety Aspects of Engineered Nanomaterials. Previously, Dr. Denison was an analyst and assistant project director in the Oceans and Environment Program, Office of Technology Assessment, United States Congress. Dr. Denison received his Ph.D. in Molecular Biophysics and Biochemistry from Yale University.

Carolyn Mattingly, PhD, received a BA in Art History from Oberlin College. Following her liberal arts education, she attended Tulane University and received a PhD in molecular toxicology. As a graduate student, she investigated the effects of the ubiquitous environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), on vertebrate development using zebrafish as model organism. She then pursued postdoctoral training at the Weill Medical College of Cornell University where she investigated the effects of retinoids on differentiation in prostate epithelial cells and mechanisms by which environmental chemicals, including AHR ligands, interfered with retinoid signaling. Since 2001, she has been an Investigator and Director of Bioinformatics at the Mount Desert Island Biological Laboratory (MDIBL) in Salisbury Cove, Maine. At MDIBL, Dr. Mattingly is involved in several collaborative research programs. First, she directs development of the publicly available Comparative Toxicogenomics Database (CTD), which aims to enhance understanding about the etiologies of environmentally influenced diseases. She also conducts a laboratory research program in which she is investigating the effects of low-level exposure to arsenic or TCDD on vertebrate development using zebrafish. Recent studies uncovered novel targets of these chemicals that make significant contributions to understanding the basis of consequent phenotypes.

Ana Navas-Acien, MD, PhD, is Assistant Professor in the Department of Environmental Health Sciences at Johns Hopkins Bloomberg School of Public Health. She is a physician-epidemiologist with a specialty in preventive medicine and public health, and a long-term interest in the health consequences of widespread environmental exposures. Based on an epidemiologic approach, her research investigates chronic health effects of arsenic, selenium, lead, cadmium, and other trace metals. Dr. Navas-Acien has served as an expert witness to the Baltimore City Council and she has served as a member of the 2010 National Toxicology Program Workshop on the Role of Environmental Chemicals in the Development of Diabetes and Obesity. She earned an MD from the University of Granada School of Medicine in Spain and a PhD in epidemiology from Johns Hopkins School of Public Health.

Chirag Patel, PhD, is Associate Professor at Harvard Medical School. His research group aims to solve problems in human health and disease by developing bioinformatics approaches to reason over large-scale environmental exposure and genomic information spanning molecules to populations. Dr. Patel received his PhD in electrical engineering from Stanford University.

Jason Richardson, MS, PhD, DABT, is Professor of Pharmaceutical Sciences and Director of the Neurodegenerative Disease Research Focus Area at the Northeast Ohio Medical University. Previously, Dr. Richardson was tenured Associate Professor and Board Certified Toxicologist in the Department of Environmental and Occupational Medicine at Rutgers Robert Wood Johnson Medical School and Resident Member of the Environmental and Occupational Health Sciences Institute. He received his M.S. (1999) and Ph.D. (2002) degrees from Mississippi State University where he conducted research on mixtures of organophosphate pesticides and the developmental neurotoxicity of organophosphates. He then completed postdoctoral training in Molecular Neuroscience at Emory University (2002-2005) where he focused on the role of pesticide exposure in Parkinson's disease. His research at EOHSI focuses on the role of environmental exposures and their interactions with genetic susceptibility as contributors to neurological disease using translational approaches. Dr. Richardson has authored or co-authored over 60 publications that have been cited over 2,000 times in the areas of developmental neurotoxicology, neurodegenerative disease, and pesticides. He has received the Outstanding New Environmental Scientist Award from the National Institute of Environmental Health Sciences and a Young Scientist Award from the American Society for Pharmacology and Experimental Therapeutics. Dr. Richardson is currently a member of the Editorial boards of Toxicological Sciences and Neurotoxicology, Neurotoxicology and Teratology, and was an Associate Editor for BMC Neurology. He has served as a grant reviewer for several NIH panels, the Michael J. Fox Foundation for Parkinson's Disease Research, Health Canada, and the United Kingdom Parkinson's Disease Society. He also served the Society of Toxicology as Secretary/Treasurer of the Neurotoxicology Specialty Section for two years.

Ivan Rusyn, MD, PhD, is Professor in the Department of Veterinary Integrative Biosciences in the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University. Prior to joining Texas A&M University, Dr. Rusyn was professor of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill. Dr. Rusyn's laboratory has an active research portfolio with a focus on the mechanisms of chemical toxicity, the genetic determinants of the susceptibility to toxicant-induced disease, and computational toxicology. His studies on health effects of chemical agents resulted in over 150 peer-reviewed publications. He has served on several US National Academies of Sciences/National Research Council committees and is currently a member of the Committee on Emerging Science for Environmental Health Decisions, Committee on Toxicology, and Committee on Incorporating 21st Century Science in Risk-Based Evaluations. He participated in WHO/IARC monographs 96, 100, 101, and 106, and chaired the overall Monograph 110, as well as chaired "Mechanistic and Other Relevant Evidence" sub-group for Monographs 101, 106, and 112. He is also serving on the Science Advisory

Board for the North Carolina Department of Environment and Natural Resources. Dr. Rusyn received his MD from Ukrainian State Medical University in Kiev and his PhD in toxicology from the University of North Carolina-Chapel Hill.

Joel Schwartz, PhD, is a Professor of Environmental Epidemiology at the Harvard School of Public Health and Director of the Harvard Center for Risk Analysis. His work has been instrumental in the removal of lead from gasoline, and the setting of particulate air pollution standards around the world. Schwartz's work tightened federal clean-air standards and improved compliance within industry. In addition to his research into lead, he was among the first to link elevated death rates to particulates of sulfur from coal-burning power plants and black carbon from motor-vehicle exhaust. Dr. Schwartz's current research interests include health consequences of exposure to pollutants, health effects of ozone exposure, and effects of antioxidants on respiratory health. Dr. Schwartz received his Ph.D. from Brandeis University.

Joyce S. Tsuji, PhD, DABT, Fellow ATS, is a Principal Scientist within the Center for Toxicology and Mechanistic Biology of Exponent's Health Sciences practice. She is a board-certified toxicologist and a Fellow of the Academy of Toxicological Sciences. Dr. Tsuji specializes in assessing exposure and risks associated with chemicals, and in communication of scientific issues. She has worked on projects in the United States and internationally for industry, trade associations, U.S. EPA and state agencies, the U.S. Department of Justice, the Australian EPA, municipalities, and private citizens. Dr. Tsuji's experience includes human health and environmental toxicology related to a wide variety of chemicals in the environment as well as in products. She has designed and directed dietary and environmental exposure studies and community programs involving heatlh eeducation and biomonitoring for populations potentially exposed to chemicals in the environment, including soil, water, and food-chain exposures. She has also assessed exposure and health risks associated with chemical exposures from air, foods, medical devices, and a variety of consumer products (e.g., cleaners, air fresheners, cosmetics, paints and coatings, carpets, glues, wood preservatives, building materials, and children's toys and play equipment), including those containing nonotechnology or nanomaterials. Dr. Tsuji has served on expert panels on toxicology and health risks issues for the National Academy of Sciences/National Research Council (including their Board on Environmental Studies and Toxicology and Committee on Toxicology), Institute of Medicine, and federal and state agencies.

Cheryl Lyn Walker, PhD, is Director of Texas A&M Health Science Center (TAMHSC) Institute of Biosciences and Technology in Houston and Welch Chair in Chemistry and a joint position as Clinical Professor in the College of Veterinary Medicine & Biomedical Sciences at Texas A&M University. Previously, Dr. Walker was Ruth and Walter Sterling Professor of Carcinogenesis at The University of Texas M.D. Anderson Cancer Center. She earned a Ph.D. in cell biology from Southwestern Medical School. Dr. Walker's research interests include studying the genetic basis of susceptibility to cancer, specifically examining the interaction of carcinogens with genes during tumor development, characterizing the effects of endocrine disruptors on human health, and developing animal models for human disease. She also studies the molecular mechanisms of kidney, breast and uterine cancers and the effect of hormones of gene expression. She has served on the Board of Scientific Counselors of the National Cancer Institute and the NIEHS National Toxicology Program, and is a past President of the Society of Toxicology.

Helmut Zarbl, PhD, is Professor of Environmental and Occupational Medicine at the Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey (UMDNJ). He is a member of the Environmental & Occupational Health Sciences Institute (EOHSI), a joint Institute of UMDNJ and Rutgers, The State University of New Jersey. He is also the Director of the NIEHS Center for

Environmental Exposures and Disease at EOHSI, is the Associate Director for Public Health Science at the Cancer Institute of New Jersey. Previously, he was a member of the Divisions of Human Biology and Public Health Sciences at the Fred Hutchinson Cancer Research Center (FHRCR), where he was Director and a Principal Investigator for the NIEHS sponsored FHFRC/University of Washington Toxicogenomics Research Consortium. Dr. Zarbl's research has focused largely on toxicogenomics and functional genomics, carcinogenesis, molecular and cellular biology, and toxicology. Specifically this has included work to understand molecular mechanisms of chemical carcinogenesis, chemoprevntion, and the genetic basis for differential susceptibility to mammary carcinogenesis using both animal and in vitro model systems. Recent studies include the role of circadian rhythm in cancer risk and prevention. His studies in the area of toxicogenomics include the development and application of standards for DNA microarray experiments, and phenotypic anchoring of response of human cells, model organisms (yeast) and target organs (rodents) to toxicants, providing insights into dose and temporal responses, as well as mechanisms of action. Dr. Zarbl is also actively involved in technology development, including his patented work on RNAi and its application to the development of novel platforms for functional genomics (with Engineering Arts, Inc). Dr. Zarbl served on the NRC committee that produced Application of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment. Previously he was an Assistant and Associate Professor at M.I.T. He earned his Ph.D. in Biochemistry from McGill University.

Lauren Zeise, PhD, Chief, Reproductive and Cancer Hazard Assessment Branch, of the California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment. In that role she oversees a variety of scientific activities concerning risk assessment, including chemical hazard and dose response assessment and development of improved methods for risk assessment. As part of Cal/EPA's environmental justice work, her group is also developing the Agency's approach to cumulative impact assessment – for characterizing the impact on communities of multiple sources of pollution and non-chemical stressors in the presence of community vulnerability. Her group works with other departments in California government in operating Biomonitoring California, the state's biomonitoring program. She co-led the team that developed California's Green Chemistry Hazard Trait regulation. Dr. Zeise has served on numerous national and international science advisory committees and boards focusing on environmental public health and improving the way chemicals are tested or evaluated for health risk. She has coauthored a number of National Academy of Science (NAS) reports, including "Science and Decisions: Advancing Risk Assessment" (2009), "Toxicity Testing in the 21st Century: A Vision and Strategy" (2007), "Sustainability and the US EPA" (2011), and "Understanding Risk: Informing Decisions in a Democratic Society" (1996). She is currently a member of the NAS committees including the Committee on Use of Emerging Science for Environmental Health Decisions. She is member, fellow, former editor and former councilor of the Society of Risk Analysis and was the 2008 recipient of the Society's Outstanding Risk Practioner Award. She is a lifetime NAS National Associate. She received her doctorate from Harvard University.

D. Upcoming Meeting

Upcoming ESEH Workshop 2016

1. **Getting the Most From Microbiome Research in the Next Decade – What Functions to Study January 14-15, 2016**

Preliminary Workshop Description

Getting the Most From Microbiome Research in the Next Decade – What Functions to Study?

The Committee's 2011 Microbiome meeting played an important role in stimulating the environmental health community to think about the microbiome, both as a modifier of exposure as a target of exposure. Since this impactful meeting, much has happened at the intersection of microbiome research and environmental health.

Microbiome research is now shifting from a focus on identifying microbiome species by 16S RNA sequencing to a recognition that studying the function rather than the genomic sequence of the microflora is likely to be more revealing. As the field shifts into studying the impact of microbiome function on disease, it is important that the environmental health community take an active role in identifying functions to be studied, as the taxonomy of microbiome functions that is described is likely to be permeate microbiome research for years to come. This meeting aims to begin the dialog about microbiome functions that are relevant to toxicology and environmental health. For example, given that the microbiome is an interface between external and internal exposure, methylation and metabolic functions may be important to examine. Along with identifying functions the toxicology community will want to see characterized, the toxicology community may also want to be involved in identifying critical windows for assessing microbiome function. For example, it may be useful to ask about microbiome function in utero or in early development, to determine the role of the microbiome in mediating early life impacts of stressor exposure. Another question is whether the microbiome is a potential mechanism for transgenerational effects of stressor exposure? Or, if circadian rhythm alterations are considered an environmental stressor, are alterations impacting the microbiome?

In short, this meeting will once again serve the important role of bringing the environmental health community and the microbiome community together, to ensure that the research on microbiome function in the next 5-10 years is conducted in a way that further advances environmental health. Important to the meeting design will be inviting keynote speaker(s) who will draw microbiome researchers and showcasing environmental health research that will inspire the next phase of microbiome research to study functions important to the environmental health community.

E. TRAVEL INFORMATION

Interindividual Variability: New Ways to Study and Implications for Decision-Making

COMMITTEE ON THE USE OF EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECICIONS

Wednesday, September 30th, 9:00 AM—5:00 PM

Thursday, October 1st, 8:30 AM— Noon*

**Committee Members & Government Liaisons will meet at this time*

MEETING SITE

The National Academies of Sciences, Engineering, and Medicine

Room 100

500 5th St. NW

Washington, DC

TRAVEL INFORMATION

If you booked your flight through Kentlands Travel and you have any emergency travel issues, please use their 24-hour emergency phone line (1-888-565-9174); you can also reach them via email at nas@uniglobekentlands.com. Please let them know that you will be traveling with NAS using travel code DELS150197.

PER DIEM ELIGIBILITY

The Academies uses a distance of 50 miles from a traveler's usual place of business to an Academies business location for per diem eligibility. Expenses including meeting related calls, internet charges, parking/tolls, taxis/metro, transportation (rail, bus), personal auto (mileage) will be reimbursed. Expenses including lodging, meals, incidentals and tips will not be reimbursed.

TRANSPORTATION EXPENSES

Transportation costs to and from the airport and the conference can be reimbursed, including taxis, super shuttle, uberX, metro bus or train, and parking garages. Parking at the NAS Building is free during the days of the workshop. Rental cars are NOT a reimbursable expense for meetings held in the Washington, DC area.

NOTE: NIH will only cover 15% tip for taxis.

PER DIEM EXPENSES

Our funders have enacted new guidelines regarding the types of charges that can be reimbursed during meetings.

- **Receipts now required for ALL expenses you are requesting reimbursement for**
- **Decrease in per diem from $71 to $53 for all travel days and the second day of the meeting**
- **Catering no longer allowed, participants must purchase meals in the NAS cafeteria (3rd floor)**

Reimbursement at per diem rate will be provided for breakfast and lunch for non-federal, non-local speakers and ESEH Committee members both days of the workshop.

September 30th Dinner: A dinner for ESEH Committee members will held at Bistro D'OC after the conclusion of the first day of the workshop. Please RSVP for dinner through the meeting participant survey. **Please bring at least $35** in cash to help speed up dinner payments.

Travelers are eligible for meal expense reimbursement beginning the date of yo ur arrival and ending on the date of your departure to your home, office, or other authorized location. The Academies must comply with federal per diem rates. The per diem rate for the upcoming workshop is $71.00/day. **However, the day of arrival in Washington, DC and the second day of the meeting (October 1st) are reimbursed at 75% per diem, which equals $53.**

NOTE: NIH will only cover 15% tip for all purchased meals.

Travelers are reimbursed for expenses incurred on travel by completing and submitting an Electronic Travel Expense Report (eTER). Instructions regarding our web -based reimbursement system will be sent directly after the meeting. To ensure that you receive your reimbursement as quickly as possible, expense reports are due within two weeks of your arrival after a meeting has taken place. You can expect reimbursement in approximately 4 -6 weeks. Contact Brendan McGovern with any questions at e -mail BMcGovern@nas.edu. Meals provided by the Academies during a meeting may not be claimed for reimbursement. **Alcohol expenses will not be reimbursed.**

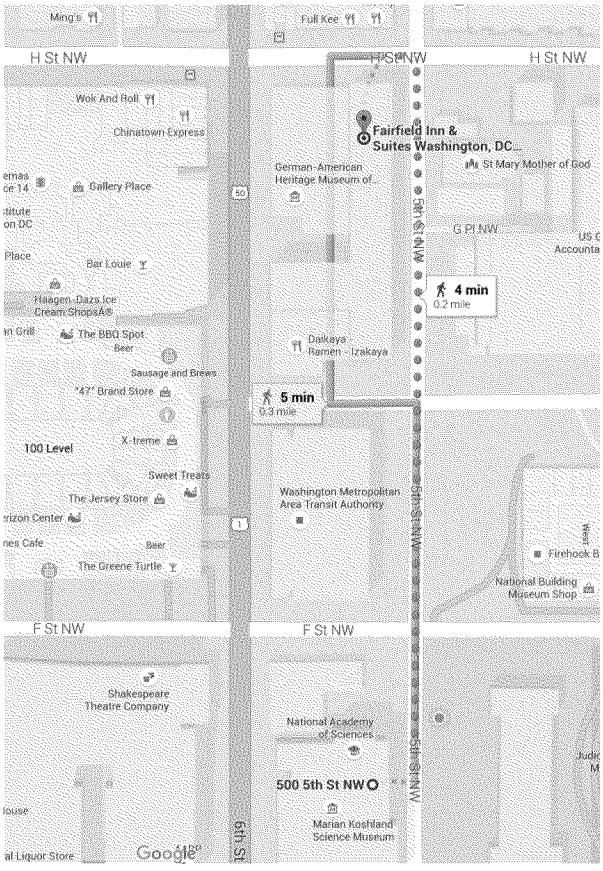
Original receipts are now required for all expenses including airfare, meals, taxi fares, parking, etc. You will be provided with instructions on how to submit an eTER to claim your reimbursements after the meeting.

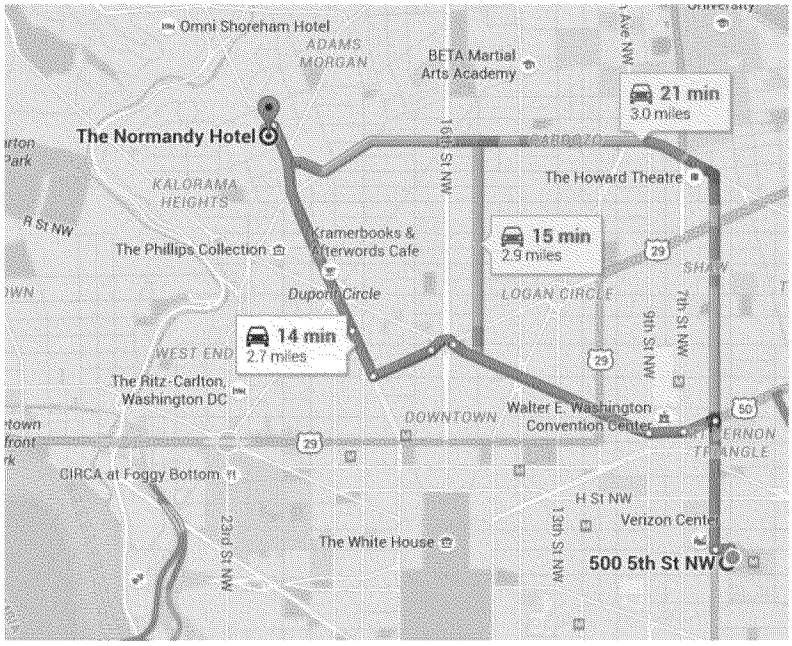
All reimbursements are limited by the maximum allowable rate under the federal per diem guidelines as established by the General Services Administration. The per diem rate for meals and incidental expenses (MI&E) for the dates of this meeting is $71. However, the day of arrival in Washington DC and the second day of the meeting are reimbursed at 75% per diem, which equals $53.

HOTEL ACCOMODATIONS

Fairfield Inn & Suites
500 H St NW
Washington, DC 20001
Telephone: (202) 697-4004

The Normandy Hotel
2118 Wyoming Ave NW
Washington, DC 20008
Telephone: (202) 483-1350





**If you booked your hotel through NAS, your hotel will have your information on file. Your hotel bill will be directly charged to NAS. The hotel may request to hold your credit card pending incidentals at checkout. Contact Kanoko Maeda at* pmaeda@nas.edu *with any questions or concerns.*

Fairfield Inn & Suites Rooming List

First Name	Last Name	Arrival Date	Departure Date	Confirmation Number
DAVID	THREADGILL	Sep-29-2015	Oct-01-2015	80607490
BARBARA	WETMORE	Sep-29-2015	Oct-01-2015	80606994
CHERYL LYN	WALKER	Sep-29-2015	Oct-01-2015	80264064
IVAN	RUSYN	Sep-29-2015	Oct-01-2015	80263336
ANA	NAVAS-ACIEN	Sep-30-2015	Oct-01-2015	80262482
CAROLYN J	MATTINGLY	Sep-30-2015	Oct-01-2015	80261712
WILLIAM H	FERLAND	Sep-29-2015	Oct-01-2015	80261104
LAUREN	ZEISE	Sep-29-2015	Oct-01-2015	80260532
HELMUT	ZARBL	Sep-29-2015	Oct-01-2015	80260078
JOYCE S	TSUJI	Sep-29-2015	Oct-01-2015	80259395
JOEL	SCHWARTZ	Sep-30-2015	Oct-01-2015	80258642
JASON	RICHARDSON	Sep-29-2015	Oct-01-2015	80257689
GARY	GINSBERG	Sep-29-2015	Oct-01-2015	80257053
JON	COOK	Sep-29-2015	Oct-01-2015	80256235
FRED	WRIGHT	Sep-30-2015	Oct-01-2015	80254992
TERRY	GORDON	Sep-29-2015	Sep-30-2015	80249703

The Normandy Hotel Rooming List

First Name	Last Name	Arrival Date	Departure Date	Confirmation Number
GINA	SOLOMON	Sep-29-2015	Oct-01-2015	64966877
MICHAEL	YUDELL	Sep-29-2015	Oct-01-2015	64966895
JOSHUA	MILLSTEIN	Sep-29-2015	Oct-01-2015	64985119

DIRECTIONS

The Keck Center, located in downtown Washington, D.C., is served by Ronald Reagan National Airport (DCA), Dulles International Airport (IAD) and Baltimore/Washington International Airport (BWI). It is accessible by Metro's Red and Green/Yellow lines.

By Car from Ronald Reagan National Airport:

1. Exit the airport to George Washington Memorial Parkway NORTH.
2. Exit to Memorial Bridge.
3. Bear LEFT after crossing Memorial Bridge into Washington, D.C.
4. Take second LEFT onto Henry Bacon Drive N.W. You must turn LEFT at this point as your route will be blocked by Jersey walls.
5. Turn RIGHT at the traffic light onto Constitution Avenue N.W.
6. Turn LEFT onto Sixth Street N.W.
7. Cross E Street N.W. and look to your right for the parking entrance immediately before the fire station.

By Car from Dulles International Airport:

1. Exit the airport to Airport Access Road EAST.
2. Follow until Access Road merges with Interstate 66 EAST.
3. Follow I-66 EAST across the Roosevelt Bridge into Washington, D.C. After the bridge, I-66 becomes Route 50 EAST/Constitution Avenue N.W.
4. Turn LEFT onto Sixth Street N.W.
5. Cross E Street N.W. and look to your right for the parking entrance immediately before the fire station.

By Car from Baltimore/Washington International Airport:

1. Exit the airport to Interstate 195 WEST.
2. Exit I-195 to MD-295 SOUTH (Baltimore-Washington Parkway) towards Washington, D.C.
3. Follow MD-295 SOUTH to exit for Route 50 WEST to downtown Washington, D.C.
4. Follow Route 50 WEST as it turns into New York Avenue N.E.
5. Turn LEFT onto Sixth Street N.W.
6. Cross F Street N.W. and look to your left for the parking entrance immediately after the fire station.

By Metro's Red Line:

1. Take Metro's Red Line to the Judiciary Square station.
2. Exit the station by following signs to the Building Museum (F Street) exit, between Fourth and Fifth Streets N.W.
3. Turn LEFT and walk WEST on F Street N.W.
4. Cross Fifth Street N.W. and turn LEFT.
5. Walk past the fire station parking lot. The next building on your right will be 500 Fifth St. N.W.

By Metro's Green or Yellow Line:

1. Take Metro's Green or Yellow Line to the Gallery Place-Chinatown station.
2. Exit the station by following signs to Seventh and F Streets/Arena.
3. Turn LEFT and walk EAST on F Street N.W., two blocks past the Verizon Center.
4. Turn RIGHT on to Fifth Street N.W.
5. Walk past the fire station parking lot. The next building on your right will be 500 Fifth St. N.W.